REMARKS

With entry of this Amendment, claims 1-56, 58-105, 107-108, 110-129, and 132-142 are pending in the application. Of those claims, claims 4-9, 11-13, 24-26, 29-32, 58, 65-66, 69, and 111-119 have been withdrawn from consideration.

Applicants thank the Examiner for the indication of allowable subject matter. Applicants herewith present new claims 144 and 145, which correspond to claims 88 and 95, respectively, rewritten in independent form. Accordingly, because claims 144 and 145 correspond to claims indicated as allowable if rewritten in independent form, claims 144 and 145 are allowable.

The Examiner indicated that the Applicants are required to provide a summary of the telephonic interview of January 15, 2004. Office Action, page 2. During that interview, several of the rejections from the Office Action mailed December 4, 2003, were discussed. Most notably, the Applicants argued that the references do not teach a third database as claimed. The Applicants also argued that the new matter rejection in that Office Action was improper. The Examiner indicated that the new matter rejection would be removed once Applicants presented a suitable argument in a response.

The Examiner has again objected to the disclosure as containing an embedded hyperlink and/or other form of browser-executable code, citing M.P.E.P. 608.01. *Id.*, page 3. Applicants have amended the specification to remove the disputed language, rendering that objection moot.

I. Objections to the Disclosure Under 35 U.S.C. § 132

The Examiner objects to the disclosure under 35 U.S.C. § 132. Office Action, paragraphs 13 and 14. Specifically, the Examiner alleges that Applicants' incorporation

by reference was improper because the application as filed does not refer to Provisional Application No. 60/130,992. The Examiner also notes that the application claims priority to Provisional Application No. 60/008,660, not 60/130,992.

Applicants herewith amend the specification to refer to Provisional Application No. 60/130,992, and submit a request for a corrected filing receipt to reflect the proper reference to the priority application. Such an amendment is allowed, even though the time period for making a claim for benefit under 37 C.F.R. § 1.78 has expired, because that time period is only applicable to applications filed on or after November 29, 2000, and the present application was filed on April 26, 2000. M.P.E.P. § 201.11(V). As such, Applicants submit that the present application now properly claims priority to Provisional Application No. 60/130,992.

The M.P.E.P. also indicates that when a benefit claim is submitted after the filing of an application, the reference to the prior application cannot include an incorporation by reference statement of the prior application unless an incorporation by reference statement of the prior application was presented upon filing of the application. M.P.E.P. § 201.11(III)(F), citing *Dart Indus v. Banner*, 636 F.2d 684, 207 USPQ 273 (C.A.D.C. 1980).

Applicants submit that in the present case, an incorporation by reference statement of the prior application was presented upon the filing of the application. The application, as filed, included the following statement regarding incorporation by reference: "[t]he present application claims the benefit of U.S. provisional application Serial No. 60/008660, filed on April 26, 1999, which is incorporated by reference herein in its entirety." Specification as filed, page 2, lines 2-4. The Examiner correctly notes

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that the reference to Provisional Application No. 60/008,660 was incorrect. However, Applicants provided the correct filing date of April 26, 1999 corresponding to Provisional Application No. 60/130,992, and indicated that the application was incorporated by reference.

In addition, Applicants submit that the inventors of the present application and Provisional Application No. 60/130,992 are identical. A copy of provisional application cover sheet for Provisional Application No. 60/130,992 is being submitted herewith to demonstrate that both the provisional application and the present application list David Manyak, Renee Zeppetello, Hao Chen, Arthur Weissman, and Garry Lang as the inventors. The cover sheet also shows that the provisional application and the present application have the same title of "Receptor Selectivity Mapping."

Moreover, Applicants submit that they clearly did not intend to refer to Provisional Application No. 60/008,660. That application was referenced in conjunction with a claim for benefit of priority. According to the filing receipt, Provisional Application No. 60/008,660 has a filing date of December 15, 1995. Obviously, Applicants cannot attempt to claim priority to a provisional application with that filing date. Provisional Application No. 60/130,992, however, has a filing date of April 26, 1999, which is exactly one year before the filing date of the present application.

An examination of the texts of the present invention and Provisional Application No. 60/130,992 also provide evidence that the incorporation by reference statement identifies Provisional Application No. 60/130,992. More particularly, many of the paragraphs in the present application are nearly identical to paragraphs present in Provisional Application No. 60/130,992. A table of paragraphs from the present

application and their corresponding paragraphs from the provisional application is

provided below:

U.S. Patent Application No. 09/558,232 (as filed)	Provisional Application No. 60/130,992
page 2, paragraph beginning at line 17	page 2, 2nd paragraph
page 3, paragraph beginning at line 18	page 2, last paragraph
page 4, paragraph beginning at line 8	page 3, 1st full paragraph
page 5, paragraph beginning at line 4	page 3, 2nd full paragraph
page 5, paragraph beginning at line 11	page 3, 3rd full paragraph
page 6, paragraph beginning at line 3	page 3, 4th full paragraph
page 6, paragraph beginning at line 11	page 3, last paragraph
page 6, paragraph beginning at line 20	page 4, 1st full paragraph
page 7, paragraph beginning at line 13	page 4, 2nd full paragraph
page 8, paragraph beginning at line 5	page 4, last paragraph page 5, 1st full paragraph
page 13, paragraph beginning at line 4	page 5, 2nd full paragraph
page 16, paragraph beginning at line 1	page 5, last paragraph
page 16, paragraph beginning at line 16	page 6, 1st full paragraph
page 17, paragraph beginning at line 19	page 16, example 1(a)
page 18, paragraph beginning at line 8	page 16, example 1(b)
page 18, paragraph beginning at line 20	page 16, example 1(c)
page 19, paragraph beginning at line 10	page 16, example 1(d)
page 19, paragraph beginning at line 17	page 17, 1st full paragraph
page 21, paragraph beginning at line 3	page 17, 2nd full paragraph

U.S. Patent Application No. 09/558,232 (as filed)	Provisional Application No. 60/130,992
page 21, paragraph beginning at line 10	page 17, 2nd full paragraph
page 22, paragraph beginning at line 4	page 17, 3rd full paragraph
page 22, paragraph beginning at line 6	page 17, 4th full paragraph
page 22, paragraph beginning at line 14	page 18, 1st full paragraph
page 24, paragraph beginning at line 18	page 18, 3rd full paragraph
page 26, paragraph beginning at line 11	page 19, 1st full paragraph
page 27, paragraph beginning at line 17	page 19, 2nd full paragraph
page 28, paragraph beginning at line 7	page 19, last paragraph
page 29, paragraph beginning at line 1	page 20, 1st paragraph (top of page)

Accordingly, because Applicants provided an incorporation by reference that correctly identified the filing date of the proper provisional application, and that provisional application has identical inventors, an identical title, and numerous sections of text that are nearly identical to the present application, Applicants contend that the incorporation by reference of Provisional Application No. 60/130,992 was proper.

For the Examiner's convenience, Applicants are providing a copy of Provisional Application No. 60/130,992.

II.   Rejections Under 35 U.S.C. § 101

The Examiner rejects claims 37, 41-49, 52-56, and 133-138 under 35 U.S.C. § 101 as being directed to non-statutory subject matter. Office Action, paragraphs 16-18.

The Examiner indicates that these claims are rejected because:

[S]aid claims are directed to a computer system, memory for storing data, and database, comprising steps for correlating data without any physical alteration step, which is considered to be non-statutory subject matter. "For example, a computer process that simply calculates a mathematical algorithm that models noise is nonstatutory. However, a claimed process for digitally filtering noise employing the mathematical algorithm is statutory." (MPEP § 2106 (IV)(B)(2)(b), part ii). Similar to the nonstatutory example above, the instant invention comprises algorithmic steps for correlating data without any physical alteration [resulting] from said analysis steps. Further, the instant invention is directed to steps for correlating data without any physical alteration of said data outside of said computer system, memory for storing data, or database.

Id., paragraph 18.

Applicants traverse the Examiner's position that claims 37, 41-49, 52-56, and 133-138 are directed to non-statutory subject matter. As noted by the Federal Circuit:

§ 101 is broad and general; its language is: "any * * * process, machine, manufacture, or composition of matter, or any * * * improvement thereof." Section 100(b) further expands "process" to include "art or method, and * * * a new use of a known process...."

State Street Bank & Trust Co. v. Signature Fin. Group, Inc., 149 F.3d 1368, 1372 (Fed. Cir. 1998).

The three unpatentable categories include: "laws of nature, natural phenomena, and abstract ideas." *Id.* at 1373 (citations omitted). According to M.P.E.P. § 2106(IV)(B)(1), "[c]laims to computer-related inventions that are clearly nonstatutory fall into the same general categories as nonstatutory claims in other arts, namely natural phenomena such as magnetism, and abstract ideas or laws of nature which constitute 'descriptive material.'"

As set forth in M.P.E.P. § 2106, "[t]he claimed invention as a whole must accomplish a practical application ... [t]hat is, it must produce a 'useful, concrete and tangible result.'" M.P.E.P. § 2106(II)(A), citing *State Street*, 149 F.3d at 1373. Further, M.P.E.P § 2106(II)(A) notes that "significant functionality [must] ... be present to satisfy

the useful result aspect of the practical application requirement [and] ... [m]erely

claiming nonfunctional descriptive material stored in a computer-readable medium does

not make the invention eligible for patenting." M.P.E.P. § 2106(II)(A) also states:

> Office personnel have the burden to establish a *prima facie*
> case that the claimed invention as a whole is directed to
> solely an abstract idea or to manipulation of abstract ideas or
> does not produce a useful result. Only when the claim is
> devoid of any limitation to a practical application in the
> technological arts should it be rejected under 35 U.S.C.
> 101...Further, when such a rejection is made, Office
> personnel must expressly state how the language of the
> claims has been interpreted to support the rejection (internal
> citations omitted).

M.P.E.P. § 2106(II)(A).

According to the Federal Circuit, the inquiry of whether a claim is statutory

focuses on "the essential characteristics of the subject matter, in particular, its practical

utility." *State Street Bank & Trust Co. v. Signature Fin. Group, Inc.*, 149 F.3d at 1375. If

a claim includes recitations that produce "a concrete, tangible and useful result," the

claim is not abstract and has practical utility. *See State Street*, 149 F.3d at 1373; *AT&T

Corp. v. Excel Communications, Inc.*, 172 F.3d 1352, 1358 (Fed. Circ. 1999), also cited

in M.P.E.P. § 2106(II)(A). And if the claim is not abstract and has practical utility, it is

statutory under 35 U.S.C. § 101. Applicants respectfully submit that claims 37, 41-49,

52-56, and 133-138 produce concrete, tangible, and useful results, and thus are

statutory.

The Examiner attempts to show that claims 37, 41-49, 52-56, and 133-138 are

non-statutory because these claims comprise "algorithmic steps for correlating data

without any physical alteration resulted from said analysis step." Office Action,

paragraph 18. The Examiner also alleges that these claims are non-statutory because

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"the instant invention is directed to steps for correlating data without any physical alteration of said data outside of said computer system, memory for storing data, or database." *Id.* Applicants disagree and submit that claims 37, 41-49, 52-56, and 133-138 are statutory for at least the following reasons.

Regarding claim 37, Applicants have amended that claim to further include "a user interface allowing a user to view information from at least one of the first database, the second database, and the third database." The inclusion of a user interface that allows a user to view information clearly places claim 37 within the realm of claims that produces "concrete, tangible and useful" results. The Examiner has already recognized that claims including a user interface to view information are statutory, as evidenced by the fact that the Examiner has not rejected any of claims 1, 33, 35, 59, 132, 139, and 142 under 35 U.S.C. § 101.

Claim 44 also includes recitations that produce "concrete, tangible and useful" results and, therefore, the claimed invention accomplishes a practical application and is not abstract. The Federal Circuit articulated in *State Street* that "the transformation of data, representing discrete dollar amounts, by a machine through a series of mathematical calculations into a final share price, constitutes a practical application of a mathematical algorithm, formula, or calculation." *See State Street*, 149 F.3d at 1601. In *AT&T Corp.*, the Federal Circuit explained that the same principles apply to method claims that do not recite a machine, stating "we consider the scope of Section 101 to be the same regardless of the form--machine or process--in which a particular claim is drafted." *AT & T Corp.* at 1357 (citations omitted).

In this case, claim 44 clearly includes a process being executed by a processor that produces useful, concrete, and tangible results. For example, the process noted in claim 44 provides, "based on one or more queries, information reflecting a relationship between a chemical compound included in the first set, a molecular target included in the second set, and the information included in the third set." The information provided by this process is evidence of a useful, concrete, and tangible result. For example, this information may be used to predict potential pharmaceutical uses of new compounds. Accordingly, a process producing such information has a useful application in the technological arts.

The recitations of claim 44 do not "simply manipulate abstract ideas"; rather, the claim recitations produce useful, concrete, and tangible results (M.P.E.P. § 2106(IV)(B)(1)) as explained above. Accordingly, this claim has practical utility and is not abstract. In addition, the subject matter recited in claim 44 clearly accomplishes a practical application within the technological arts.

Moreover, claim 44 is not directed to the "manipulation of an abstract idea." Because claim 44 is not abstract, the claim does not merely recite "nonfunctional descriptive matter." According to M.P.E.P. § 2106(IV)(B)(1), nonfunctional descriptive material "includes but is not limited to music, literary works and a compilation or mere arrangement of data." A memory that stores data for access by a process, which provides "based on one or more queries, information reflecting a relationship between a chemical compound included in the first set, a molecular target included in the second set, and the information included in the third set," where "the information reflecting the relationship is relevant to a predictability of a potential use of a new compound," as

recited in claim 44, is not a mere arrangement of data, not abstract, and therefore statutory under 35 U.S.C. § 101.

Claims 45, 46, 54, and 133 (as amended) are drawn to a memory, database, storage device, and memory, respectfully, that are each accessible by processes producing information associated with data stored in the respective memory, database, storage device, and memory. Applicants respectfully submit that these claims are statutory for reasons similar to those provided for claim 44 above.

The Examiner rejected claims 41-43, 47-49, 52-53, 55-56, and 134-138 under 35 U.S.C. § 101, due to their dependence on similarly rejected independent claims. Because independent claims 37, 44-46, 54, and 133 are statutory for the reasons set forth above, Applicants submit that dependent claims 41-43, 47-49, 52-53, 55-56, and 134-138 are also statutory.

For at least the foregoing reasons, Applicants request that the Examiner withdraw the rejection of claims 37, 41-49, 52-56, and 133-138 under 35 U.S.C. § 101.

III.   Rejections Under 35 U.S.C. § 112, First Paragraph

The Examiner rejects claims 44, 46, 47, 54-56, 60, 61, 63, 64, 67, 68, 77, 78, 87, 89, 96, 102, 108, 110, and 132-141 under 35 U.S.C. § 112, first paragraph, as containing new matter. Office Action, paragraphs 19-28.

Regarding claims 44, 46, 47, and 139-141, the Examiner indicates that these claims were rejected because the specification does not support "new information" or "new relationship." *Id.*, paragraph 24. Applicants dispute the Examiner's allegation and remind the Examiner that it was the Examiner's suggestion along with Examiner Ardin Marschel, at the personal interview conducted on February 3, 2004, to include claims

with similar language. However, in order to expedite prosecution, Applicants have amended claims 44, 46, and 139-141 to remover reference to "new information" or a "new relationship." Applicants further have amended independent claims 44, 46, and 139 to refer to information that "is relevant to a predictability of a potential use of a new compound." Support for this feature may be found, for example, in the specification at page 32, lines 14-15. Accordingly, claims 44, 46, 47, and 139-141 do not contain new matter.

Regarding claims 54-56, the Examiner indicates that the feature of "creating a full-rank data set of test results" has not been found in the specification. Office Action, paragraph 25. Applicants disagree. The specification provides explicit support for such a feature. For example, the specification, at page 29, lines 20-21, reads "in table 220 screening results are entered as a numerical descriptor identifying the potency or magnitude of the binding or other effect...for each of a plurality of chemical compounds tested against each of a plurality of molecular targets." In further describing this table, the specification at page 30, lines 2-4 reads "all such matrix points for chemicals x targets in tables 210 and 220 are determined and entered into the database such that a full-rank dataset is derived." At least these sections of the specification provide support for the feature of "creating a full-rank data set of test results."

Further with respect to claims 54-56 and 132, the Examiner indicates that the feature of "all possible combinations of the compounds selected" has not been found in the specification. Office Action, paragraph 26. Applicants again disagree. The specification provides support for this feature at page 26, lines 3-4, which reads, "[t]ables 500 and 600 together may be a full-rank database (e.g., including all possible

combinations between compounds and molecular targets in a relational database system)..." For the reasons set forth above, Applicants request that the Examiner withdraw the rejection of claims 54-56 and 132, because the disputed language of the claims does not constitute new matter.

Regarding claims 60, 63, and 137, Applicants have removed the term "all or substantially" from these claims. Applicants respectfully submit that the new matter rejection of claims 60, 63, and 137 has been overcome.

Regarding claims 61, 64, and 138, the Examiner indicates that these claims were rejected because the feature of "a majority of a plurality of compounds" has not been found in the specification. Office Action, paragraph 27. Applicants respectfully submit that the specification, at page 29, lines 15-19, indicates that a table may store screening results "for each of a plurality of chemical compounds tested against each of a plurality of molecular targets..." As further disclosed by the specification, "[i]n a preferred embodiment, all such matrix points for chemicals x targets in tables 210 and 220 are determined and entered into the database such that a full-rank dataset is derived." Specification, page 30, lines 2-4. A table that stores results for all matrix points necessarily includes results corresponding to a majority of compounds and a majority of molecular targets.

In addition, as noted, a full-rank dataset is only one preferred embodiment. The specification more generally indicates that results may be stored for each of a *plurality* of chemical compounds tested against each of a *plurality* of molecular targets. Because plurality means more than one and because the specification discloses that plurality can mean as much as "all," it follows that plurality may mean any number that is more than

one up to and including all of the compounds or targets. Accordingly, the specification provides support for "a majority of the compounds." For the reasons set forth above, Applicants request that the Examiner withdraw the rejection of claims 61, 64, and 138, because the disputed language of the claims does not constitute new matter.

Regarding claims 67, 68, 77, 78, 87, 89, 96, 102, 108, and 110, the Examiner indicates that those claims were rejected because the incorporation by reference of U.S. Provisional Application No. 60/130,992 was improper. Office Action, paragraphs 22-23. Applicants refer the Examiner to the argument presented above in Section I with respect to the outstanding objections to the disclosure under 35 U.S.C. § 132. This argument establishes that U.S. Provisional Application No. 60/130,992 was properly incorporated by reference. Applicants respectfully submit that the disputed subject matter of claims 67, 68, 77, 78, 87, 89, 96, 102, 108, and 110 may be found in Provisional Application No. 60/130,992, filed April 26, 1999, upon which the Applicants claim priority for this application. Subject matter from the Provisional Application includes the additional sheets of drawings attached to the Amendment of September 10, 2003. For the reasons set forth above, Applicants request that the Examiner withdraw the rejection of claims 67, 68, 77, 78, 87, 89, 96, 102, 108, and 110 because the disputed language of the claims does not constitute new matter.

Regarding claim 133, the Examiner indicates that this claim is rejected because the limitations of "identified chemical compounds," and "identified molecular targets" are not in the specification. *Id.*, paragraph 28. Applicants respectfully disagree. Claim 133 specifies a data structure for maintaining information identifying a plurality of chemical compounds and a plurality of molecular targets. The specification supports those

features. For example, according to the present invention, data corresponding to compounds and records may be stored in records. These records may include various information associated with the different compounds and targets, such as name, type, etc. The information stored in those records effectively act to identify the different compounds and targets. The disputed limitations of "identified chemical compounds" and "identified molecular targets" have proper antecedent basis (e.g., "a data structure for maintaining information identifying a plurality of chemical compounds and a plurality of molecular targets..." provides proper antecedent basis). Because the specification has support for maintaining this type of information, the specification also necessarily supports the disputed limitations. For these reasons, Applicants request that the Examiner withdraw the rejection of claim 133. Moreover, Applicants request that the Examiner withdraw the rejections of claims 134-138 for reasons similar to those provided above for claim 133.

Applicants also note that claims 77, 87, and 110, are only rejected under 35 U.S.C. § 112, first paragraph. Because the rejections to these claims under 35 U.S.C. § 112, first paragraph, have been overcome for the reasons specified above, Applicants submit these claims are allowable.

IV.   Rejections Under 35 U.S.C. § 103

A.   Goto et al. taken with Bult et al. in combination with Antman et al.

The Examiner rejects claims 1-3, 14-23, 27-28, 33-56, 59-64, 70-76, 78, 80, 89-91, 93-94, 97-105, 120-121, 124-125, 127-129, and 132-142 under 35 U.S.C. § 103(a) as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. Office Action, paragraph 31. This rejection is respectfully traversed because a

prima facie case of obviousness has not been made by the Examiner. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, the prior art reference as modified must teach or suggest all the claim elements. Second, there must be some suggestion or motivation, either in the reference or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine the reference teachings. Third, a reasonable expectation of success must exist. Moreover, each of these requirements must "be found in the prior art, and not be based on applicant's disclosure." (M.P.E.P. § 2143.03 (8th ed. 2001)).

Applicants submit that Bult et al. is not prior art with respect to the present application. The Examiner indicates that Bult et al. was published in May 1999. The present application, however, has a priority date of April 26, 1999, which is earlier than May 1999. Accordingly, only Goto et al. and Antman et al. remain as applicable references with respect to claims 1-3, 14-23, 27-28, 33-56, 59-64, 70-76, 78, 80, 89-91, 93-94, 97-105, 120-121, 124-125, 127-129, and 132-142.

Applicants note that Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. However, none of independent claims 1, 33, 35, 37, 44, 45, 46, 54, 59, 132, 133, and 139, which have each been rejected as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al., include this feature. Applicants request clarification on how Antman et al. applies to claims 1, 33, 35, 37, 44, 45, 46, 54, 59, 132, 133, and 139.

Present claim 1 provides for a computer system comprising: a first database containing records corresponding to a plurality of chemical compounds and records

corresponding to biological information related to effects of such chemical compounds on biological systems; a second database containing records corresponding to a plurality of molecular targets; a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target; and a user interface allowing a user to view the selected compound and to selectively view information from the first database, the second database, and the third database as it relates to a compound record in the first database or as it relates to a molecular target in the second database.

Applicants respectfully submit that Goto et al. in view of Bult et al. and Antman et al. do not disclose (or suggest) this claimed combination of elements. For example, the references do not disclose or suggest at least a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target.

Goto et al. discloses the LIGAND chemical database, which includes two sections: ENZYME and COMPOUND (page 591, left col.). The COMPOUND section

has information on the nomenclature and chemical structures of compounds (page 591, left col.). The ENZYME section of the LIGAND database accumulates information on known enzymes and reactions (page 592, right col.). Reaction data is reflected in the REACTION field of the ENZYME entry (page 592, right col.). Enzymatic or non-enzymatic reactions may be written in the form of a chemical reaction in the REACTION field and maintained in a relational database as a substrate-product binary relationship, or a set of substrate-product binary relationships (page 594, left col.). Goto et al. also discloses a database called BRITE that is for molecular interactions in general (page 597, right col.). LIGAND is a component of the KEGG and DBGET/LinkDB systems.

In contrast, systems and methods consistent with the present invention as recited, for example, in present claim 1 provide for a database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in a first database and each of a plurality of molecular targets in a second database. The tests include information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and the selected molecular target.

The databases disclosed in Goto et al. do not store information that reflects the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and the selected molecular target. Goto et al. shows interaction information in general but not information on the effect that a selected compound has on an interaction between a reference compound and a selected molecular target.

Applicants respectfully submit that the Examiner neglects to address this feature at all. Instead, the Examiner appears to concentrate on whether the cited references teach screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of compounds in the second database. See, e.g., Office Action, paragraphs 32-38. The Examiner never even attempts to show how the cited references allegedly teach storing information that reflects the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and the selected molecular target. Applicants submit that Goto et al. does not provide such a teaching.

Moreover, despite the Examiner's argument to the contrary, Goto et al. does not disclose a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database. KEGG and affiliated databases discussed in Goto et al. are designed to incorporate individual components of *natural* biological systems in order to define cellular pathways present in nature. The relationships among the components are made by association, not by measurement. In contrast, the present invention involves the creation of a third database comprising measurements or tests made in a laboratory of interactions between each of the chemicals in a first database and each of the targets in the second database. A primary purpose of this third database is for use in drug discovery and development, including use for identifying or optimizing new drug candidates, not for elucidation of natural biological pathways in cells as described in KEGG.

More particularly, the Examiner alleges that Goto et al. discloses it is possible to generate all possible paths for all compounds, pointing to page 596 of Goto et al. in doing so. Office Action, paragraphs 34 and 38. The purpose of the components of KEGG is to delineate natural pathways of interconnected enzymes, one example of which is the well-known Krebs Cycle. In such a cyclic pathway, one chemical compound interacts with both the preceding (as product) and succeeding enzyme (as substrate) or target in the pathway. Eventually, the cycle is completed by the "last" product in the pathway acting as the substrate for the "first" enzyme in the pathway. In this regard, it does represent an interaction between one chemical and each of a plurality (two) of targets. It does not, however, represent the interaction of *each of a plurality* of compounds in the pathway (substrates/products) and *each of a plurality* of targets (enzymes) in the pathway. Nor would one skilled in the art find it obvious to do so from Goto et al., because the purpose of KEGG and associated databases in Goto et al. is to elucidate natural pathways which by the nature of the term pathway indicates linear, or at best branched, sequences of interacting molecules, not testing the full set of interactions of each compound and each target.

In the context of Goto et al., the statement, "it is possible to generate all possible paths starting and ending at all compounds," simply means that one can identify (to the extent that the information was contained in the KEGG database) any linear pathway of which any compound in the KEGG database is a component by a search mechanism. One cannot contemplate, however, generating all possible target interactions for all compounds in the database because KEGG does not include or envision a third

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database that includes tests of interactions between each of the plurality of compounds in the first database and each of the plurality of targets in the second database.

Furthermore, in systems consistent with the present invention, interactions between chemicals and targets are systematically determined by _testing_ for interactions, for example to obtain screening results. The Examiner has incorrectly used or interpreted the term "screen results" or "screening results." For example, the Examiner's statement, in paragraph 38 of the Office Action, that "Goto et al. identifies new chemical compounds (screen results) appearing in these reactions and [adds] them as new COMPOUND entries," does not correctly correspond to the "screening results" of the present invention. Goto et al. describes COMPOUND (LIGAND/COMPOUND) as a database containing "Chemical compounds in living organisms (Goto et al. page 598, Table 5), allowing natural pathways appearing in KEGG (including via LIGAND) to be _searched_ for the possible presence of such chemicals that are reported in KEGG to be components of known documented pathways. KEGG/PATHWAY is defined as "Metabolic and regulatory pathways" (Goto et al., page 598, Table 5). There is no indication of any testing for interactions to, for example, obtain screening results.

Accordingly, Goto et al. does not teach or suggest "a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target."

Antman et al. is not sufficient to overcome the deficiencies of Goto et al. Antman et al. discusses compounds tested for their clinical effects on patients, including adverse effects. Antman et al. does not discuss the effect of such compounds on molecular targets, only on clinical outcome. Furthermore, the compounds are tested individually on patients, so that there is no testing of the interactions of each of a plurality of compounds on each of a plurality of molecular targets. Additionally, as noted above, Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. Accordingly, Antman et al. does not teach or suggest "a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target."

Moreover, there is no suggestion or motivation to combine or otherwise modify the cited references in a way that shows the claimed invention. The Examiner asserts that one of ordinary skill in the art would have been motivated "to build 'better databases' by integrating the COMPOUND database (first database} of KEGG with data from drug treatments (compounds) failed in human clinical tests as disclosed by Antman et al." Office Action, paragraph 61. However, Goto et al. is not concerned with creating "better databases" for clinical experts. Accordingly, there is no need for Goto et al. to require the data disclosed by Antman et al.

Determinations of *prima facie* obviousness must be supported by a finding of "substantial evidence." *See In re Zurko*, 258 F.3d 1379, 1386 (Fed. Cir. 2001). Specifically, unless "substantial evidence" found in the record supports the factual determinations central to the issue of patentability, including motivation, the rejection is improper and should be withdrawn. Further, "[o]bviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art." *See M.P.E.P.* § 2143.01.

In this case, there is no "substantial evidence" in the record or in the art to support the attempted combination of Goto et al. and Antman et al., and the requisite motivation required to support a *prima facie* case of obviousness is lacking. The Examiner has not established, by substantial evidence, that a skilled artisan having the art before him would have been motivated to combine the teachings of Goto et al. with Antman et al. in a manner resulting in Applicants' claimed invention. As explained, there is no motivation for Goto et al. to require the data disclosed by Antman et al. because Goto et al. is not concerned with creating "better databases" for clinical experts.

Further evidence of nonobviousness exists in Goto et al., which admits that:

> the knowledge of molecular interactions will remain fragmentary because of the lack of technologies for systematic experiments to detect molecular interactions, except for the yeast two-hybrid system for protein-protein interactions. It is a grand challenge in bioinformatics to predict molecular interactions, including those involving small substances, as a step toward understanding molecular wiring diagrams of life.

> Goto et al., pages 597-598.

In contrast to the state of the art at the time, Applicants created the presently claimed third database of tests of interactions, including those involving small substances for use in prediction of other molecular interactions. The present invention, however, is primarily directed toward prediction of features relevant to drug discovery and development, rather than the primary utility of Goto et al., which was understanding natural pathways or molecular wiring diagrams of life.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 1 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al. Because independent claims 33, 37, 133, 139, and 142 recite language similar to that which distinguishes claim 1 from Goto et al., Bult et al., and Antman et al., Applicants further submit that claims 33, 37, 133, 139, and 142 are patentable over Goto et al. taken with Bult et al. in combination with Antman et al. for at least the reasons given with respect to claim 1.

Present claim 35 provides for a computer system comprising: a first database containing data corresponding to a plurality of chemical compounds and data corresponding to biological information related to effects of such chemical compounds on biological systems; a second database containing data corresponding to a plurality of molecular targets; a third database containing data corresponding to tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database; and a user interface allowing a

user to view data from the first database, the second database, and the third database as it relates to at least one compound in the first database or as it relates to at least one molecular target in the second database or as it relates to one or more interactions in the third database.

Applicants respectfully submit that Goto et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least this claimed combination of elements. For example, the references do not disclose or suggest at least a third database containing data corresponding to tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database.

As explained above with reference to the discussion on claim 1, Goto et al. does not include or envision a third database that includes tests of interactions between each of the plurality of compounds in the first database and each of the plurality of targets in the second database.

Accordingly, Goto et al. does not teach or suggest "a third database containing data corresponding to tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database."

Antman et al. is not sufficient to overcome the deficiencies of Goto et al. Antman et al. discusses compounds tested for their clinical effects on patients, including adverse effects. Antman et al. does not discuss the effect of such compounds on molecular targets, only on clinical outcome. Furthermore, the compounds are tested individually on patients, so that there is no testing of the interactions of each of a plurality of compounds on each of a plurality of molecular targets. Additionally, as noted above, Antman et al. was only used by the Examiner to allege a teaching of a first database of

chemical compounds that have failed in preclinical or human clinical tests. Accordingly, Antman et al. does not teach or suggest "a third database containing data corresponding to tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database."

Moreover, there is no suggestion or motivation to combine or otherwise modify the cited references in a way that shows the claimed invention, as explained above with reference to the discussion on claim 1.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 35 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al. Because independent claim 59 recites language similar to that which distinguishes claim 35 from Goto et al., Bult et al., and Antman et al., Applicants further submit that claim 59 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al. for at least the reasons given with respect to claim 35.

Present claim 44 provides for a memory for storing data for access by a process being executed by a processor, the memory comprising: a data structure for maintaining (i) a first set of information associated with one or more chemical compounds, (ii) a second set of information associated with one or more molecular targets, and (iii) a third set of information reflecting interactions between each of a plurality of the chemical compounds and each of a plurality of the molecular targets, wherein the process may provide, based on one or more queries, information reflecting

a relationship between a chemical compound included in the first set, a molecular target included in the second set, and the information included in the third set, and wherein the information reflecting the relationship is relevant to a predictability of a potential use of a new compound.

Applicants respectfully submit that Goto et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least this claimed combination of elements. For example, the references do not disclose or suggest at least a third set of information reflecting interactions between each of a plurality of the chemical compounds and each of a plurality of the molecular targets, wherein the process may provide, based on one or more queries, information reflecting a relationship between a chemical compound included in the first set, a molecular target included in the second set, and the information included in the third set, and wherein the information reflecting the relationship is relevant to a predictability of a potential use of a new compound.

As explained above with reference to the discussion on claim 1, Goto et al. does not include or envision a third database that includes tests of interactions between each of the plurality of compounds in the first database and each of the plurality of targets in the second database.

In addition, Goto et al. does not teach that a process may provide, based on one or more queries, information reflecting a relationship between a chemical compound included in the first set, a molecular target included in the second set, and the information included in the third set, and wherein the information reflecting the relationship is relevant to a predictability of a potential use of a new compound. Nothing in Goto et al. provides a similar function.

Accordingly, Goto et al. does not teach or suggest "a third set of information reflecting interactions between each of a plurality of the chemical compounds and each of a plurality of the molecular targets, wherein the process may provide, based on one or more queries, information reflecting a relationship between a chemical compound included in the first set, a molecular target included in the second set, and the information included in the third set, and wherein the information reflecting the relationship is relevant to a predictability of a potential use of a new compound."

Antman et al. is not sufficient to overcome the deficiencies of Goto et al. Antman et al. discusses compounds tested for their clinical effects on patients, including adverse effects. Antman et al. does not discuss the effect of such compounds on molecular targets, only on clinical outcome. Furthermore, the compounds are tested individually on patients, so that there is no testing of the interactions of each of a plurality of compounds on each of a plurality of molecular targets. Additionally, as noted above, Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. Accordingly, Antman et al. does not teach or suggest "a third database containing data corresponding to tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database."

Moreover, there is no suggestion or motivation to combine or otherwise modify the cited references in a way that shows the claimed invention, as explained above with reference to the discussion on claim 1.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the

art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 44 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al.

Present claim 45 provides for the following: in a system for correlating data associated with chemical compounds and molecular targets, a memory comprising: a first array of records, each including information indicative of a chemical compound; a second array of records, each including information indicative of a molecular target; a third array of records, each corresponding to a binding capability between each of the chemical compounds and molecular targets; and a fourth array of records, each corresponding to a biological activity related to the chemical compounds and the molecular targets, wherein a process may access the first, second, and third arrays to produce information corresponding to a drug potential for a new compound based on relationships between characteristics associated with the new compound and a selected biological activity included in the fourth array of records or patterns of binding capabilities included in the third array.

Applicants respectfully submit that Goto et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least this claimed combination of elements. For example, the references do not disclose or suggest at least a third array of records, each corresponding to a binding capability between each of the chemical compounds and molecular targets. The references also do not disclose or suggest that a process may access the first, second, and third arrays to produce information corresponding to a drug potential for a new compound based on relationships between characteristics

associated with the new compound and a selected biological activity included in the fourth array of records or patterns of binding capabilities included in the third array.

The databases disclosed in Goto et al. do not include an array of records, each corresponding to a binding capability between _each of_ the chemical compounds and molecular targets. Assuming, arguendo, that a database in Goto et al. can be considered to store data related to binding capability related to some chemical compounds and molecular targets, the data does reflect a binding capability between _each of_ the chemical compounds and molecular targets. Accordingly, Goto et al. does not teach or suggest "a third array of records, each corresponding to a binding capability between each of the chemical compounds and molecular targets."

Moreover, Goto et al. does not teach that a process may access the first, second, and third arrays to produce _information corresponding to a drug potential for a new compound based on relationships between characteristics_ associated with the new compound and a selected biological activity included in the fourth array of records or patterns of binding capabilities included in the third array. Nothing in Goto et al. provides a similar function. Accordingly, Goto et al. does not teach or suggest that "a process may access the first, second, and third arrays to produce information corresponding to a drug potential for a new compound based on relationships between characteristics associated with the new compound and a selected biological activity included in the fourth array of records or patterns of binding capabilities included in the third array."

Antman et al. is not sufficient to overcome the deficiencies of Goto et al. Antman et al. discusses compounds tested for their clinical effects on patients, including adverse

effects. Antman et al. does not discuss the effect of such compounds on molecular targets, only on clinical outcome. Furthermore, the compounds are tested individually on patients, so that there is no testing of the binding capability of each of a plurality of compounds on each of a plurality of molecular targets. Additionally, as noted above, Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. Accordingly, Antman et al. does not teach or suggest "a third array of records, each corresponding to a binding capability between each of the chemical compounds and molecular targets." Antman et al. also does not teach that "a process may access the first, second, and third arrays to produce information corresponding to a drug potential for a new compound based on relationships between characteristics associated with the new compound and a selected biological activity included in the fourth array of records or patterns of binding capabilities included in the third array."

Moreover, there is no suggestion or motivation to combine or otherwise modify the cited references in a way that shows the claimed invention, as explained above with reference to the discussion on claim 1.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 45 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al.

Present claim 46 provides for the following: a database for storing data for access by a process executed by a processor, the database comprising: a compound

data structure including data associated with a set of chemical compounds; a target data structure including data associated with a set of molecular targets; and a result data structure including data corresponding to results of screening tests between each of a plurality of chemical compounds from the set of chemical compounds and each of a plurality of molecular targets from the set of molecular targets, wherein the process determines information reflecting a relationship between the data included in the compound, target, and result data structures, and wherein the information reflecting the relationship is used to validate disease relevance or biological function of a new molecular target.

Applicants respectfully submit that Goto et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least this claimed combination of elements. For example, the references do not disclose or suggest at least a result data structure including data corresponding to results of screening tests between each of a plurality of chemical compounds from the set of chemical compounds and each of a plurality of molecular targets from the set of molecular targets, wherein the process determines information reflecting a relationship between the data included in the compound, target, and result data structures, and wherein the information reflecting the relationship is used to validate disease relevance or biological function of a new molecular target.

As explained above with reference to the discussion on claim 1, Goto et al. does not include or envision a third database that includes tests of interactions between each of the plurality of compounds in the first database and each of the plurality of targets in the second database.

In addition, Goto et al. does not teach that a process determines information reflecting a relationship between the data included in the compound, target, and result data structures, and wherein the information reflecting the relationship is used to validate disease relevance or biological function of a new molecular target. Nothing in Goto et al. provides a similar function.

Accordingly, Goto et al. does not teach or suggest "a result data structure including data corresponding to results of screening tests between each of a plurality of chemical compounds from the set of chemical compounds and each of a plurality of molecular targets from the set of molecular targets, wherein the process determines information reflecting a relationship between the data included in the compound, target, and result data structures, and wherein the information reflecting the relationship is used to validate disease relevance or biological function of a new molecular target."

Antman et al. is not sufficient to overcome the deficiencies of Goto et al. Antman et al. discusses compounds tested for their clinical effects on patients, including adverse effects. Antman et al. does not discuss the effect of such compounds on molecular targets, only on clinical outcome. Furthermore, the compounds are tested individually on patients, so that there is no testing of the interactions of each of a plurality of compounds on each of a plurality of molecular targets. Additionally, as noted above, Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. Accordingly, Antman et al. does not teach or suggest "a result data structure including data corresponding to results of screening tests between each of a plurality of chemical compounds from the set of chemical compounds and each of a plurality of molecular

targets from the set of molecular targets, wherein the process determines information reflecting a relationship between the data included in the compound, target, and result data structures, and wherein the information reflecting the relationship is used to validate disease relevance or biological function of a new molecular target."

Moreover, there is no suggestion or motivation to combine or otherwise modify the cited references in a way that shows the claimed invention, as explained above with reference to the discussion on claim 1.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 46 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al.

Present claim 54 provides for the following: in a system for maintaining test screening results, a storage device for storing data for access by a process being executed by a processor comprising: a data set including information corresponding to results of screening assay tests that measure an interaction between all possible combinations of chemical compounds in a compound set and molecular targets in a molecular target set, thereby creating a full-rank data set of test results, wherein the process provides selected result information based on a request associated with a selected chemical compound or molecular target. Nor is there any other database disclosed by Goto et al. that suggests storing such information.

Applicants respectfully submit that Goto et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least this claimed combination of elements. For

example, the references do not disclose or suggest at least a data set including information corresponding to results of screening assay tests that measure an interaction between all possible combinations of chemical compounds in a compound set and molecular targets in a molecular target set, thereby creating a full-rank data set of test results.

The databases disclosed in Goto et al. do not store information corresponding to results of screening assay tests that measure an interaction between *all possible combinations* of chemical compounds in a compound set and molecular targets in a molecular target set. Goto et al. does show some interaction information. For example, as noted above, the BRITE database stores information on molecular interactions in general (page 597, right col.). This information on molecular interactions, however, does not teach the concept of storing information corresponding to results of screening assay tests that measure an interaction between *all possible combinations* of chemical compounds in a compound set and molecular targets in a molecular target set.

The Examiner alleges that Goto et al. discloses it is possible to generate all possible paths for all compounds, pointing to page 596 of Goto et al. in doing so. Office Action, paragraphs 34 and 38. The purpose of these components of KEGG is to delineate natural pathways of interconnected enzymes, one example of which could be the well-known Krebs Cycle. In such a cyclic pathway, one chemical compound interacts with both the preceding (as product) and succeeding enzyme (as substrate) or target in the pathway. Eventually, the cycle is completed by the "last" product in the pathway acting as the substrate for the "first" enzyme in the pathway. In this regard, it does represent an interaction between one chemical and each of a plurality (two) of

targets. It does not, however, represent an interaction between *all possible combinations* of chemical compounds in a compound set and molecular targets in a molecular target set.

Accordingly, Goto et al. does not teach or suggest "a data set including information corresponding to results of screening assay tests that measure an interaction between all possible combinations of chemical compounds in a compound set and molecular targets in a molecular target set, thereby creating a full-rank data set of test results."

Antman et al. is not sufficient to overcome the deficiencies of Goto et al. Antman et al. discusses compounds tested for their clinical effects on patients, including adverse effects. Antman et al. does not discuss the effect of such compounds on molecular targets, only on clinical outcome. Furthermore, the compounds are tested individually on patients, so that there is no testing of the interactions between *all possible combinations* of chemical compounds in a compound set and molecular targets in a molecular target set. Additionally, as noted above, Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. Accordingly, Antman et al. does not teach or suggest "a data set including information corresponding to results of screening assay tests that measure an interaction between all possible combinations of chemical compounds in a compound set and molecular targets in a molecular target set, thereby creating a full-rank data set of test results."

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the

art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 54 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al. Because independent claim 132 recites language similar to that which distinguishes claim 54 from Goto et al., Bult et al., and Antman et al., Applicants further submit that claim 132 is patentable over Goto et al. taken with Bult et al. in combination with Antman et al. for at least the reasons given with respect to claim 54.

Dependent claims 2-3, 14-23, 27-28, 34, 36, 38-54, 55-56, 60-64, 70-76, 78, 80, 89-91, 93-94, 97-105, 120-121, 124-125, 127-129, 134-138, and 140-141 are allowable not only for the reasons stated above with regard to their respective allowable base claims, but also for their own additional features that distinguish them from Goto et al., Bult et al, and Antman et al.

In view of these remarks, Applicants request that the Examiner withdraw the rejection.

B. Ogata et al. taken with Bult et al. in combination with Antman et al.

The Examiner rejects claims 1, 10, 17, 59, 67-68, 79, 81-86, 92, 108, 122, and 123 under 35 U.S.C. § 103(a) as being unpatentable over Ogata et al. taken with Bult et al. in combination with Antman et al. Office Action, paragraph 62. Applicants traverse the rejection.

As noted above, Bult et al. is not prior art with respect to the present application. Accordingly, only Ogata et al. and Antman et al. remain as applicable references with respect to claims 1, 10, 17, 59, 67-68, 79, 81-86, 92, 108, 122, and 123.

Applicants note that Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. However, none of independent claims 1 and 59, which have each been rejected as being unpatentable over Ogata et al. taken with Bult et al. in combination with Antman et al., include this feature. Applicants request clarification on how Antman et al. applies to claims 1 and 59.

Applicants respectfully submit that Ogata et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least the claimed combination of elements of claim 1. For example, as in the case of rejections involving Goto et al., the references do not teach or suggest "a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target."

Ogata et al. discloses that KEGG maintains a catalog of chemical elements, compounds, and other substances in living cells as the LIGAND database (page 29, right col.). Ogata et al. discloses that the LIGAND database stores information of chemical compounds, enzyme molecules, and enzymatic and non-enzymatic reactions (page 33, right col.).

Systems and methods consistent with the present invention as recited in present claim 1 provide for a database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in a first database

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and each of a plurality of molecular targets in a second database. The tests include information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and the selected molecular target.

The databases disclosed in Ogata et al. do not store information that reflects the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and the selected molecular target. Ogata et al. shows interaction information in general but not information on the effect that a selected compound has on an interaction between a reference compound and a selected molecular target.

Applicants respectfully submit that the Examiner neglected to address this feature at all. The Examiner never even attempts to show how the cited references allegedly teach storing information that reflects the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and the selected molecular target. Applicants submit that Ogata et al. does not provide such a teaching.

As mentioned by the Examiner, Ogata et al. is similar to Goto et al., in that both references discuss KEGG and other associated databases. As such, Applicants submit that Ogata et al. does not disclose other features of claim 1 not specifically discussed above for reasons similar to those provided with respect to Goto et al. in section A.

Accordingly, Ogata et al. does not teach or suggest "a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets

in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target."

Antman et al. is not sufficient to overcome the deficiencies of Ogata et al. for reasons similar to those provided above with respect to Goto et al. in section A. Moreover, for reasons similar to those provided above with respect to Goto et al. in section A, there is no suggestion or motivation to combine or otherwise modify Ogata et al. and Antman et al. in a way that shows the claimed invention.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 1 is patentable over Ogata et al. taken with Bult et al. in combination with Antman et al.

Applicants also submit that Ogata et al. in view of Bult et al. and Antman et al. do not disclose or suggest at least the claimed combination of elements of claim 59. For example, as in the case of rejections involving Goto et al., the references do not teach or suggest "a third database containing records corresponding to the results of tests to determine the interaction between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database."

As similarly explained with reference to Goto et al., KEGG and affiliated databases discussed in Ogata et al. are designed to incorporate individual components of *natural* biological systems in order to define cellular pathways present in nature. The

relationships among the components are made by association, not by measurement. In contrast, the present invention involves the creation of a third database comprising measurements or tests made in a laboratory of interactions between each of the chemicals in a first database and each of the targets in the second database. A primary purpose of this third database is for use in drug discovery and development, including use for identifying or optimizing new drug candidates, not for elucidation of natural biological pathways in cells as described in KEGG.

The Examiner refers to paragraphs 33-39 of the Office Action, which discuss Goto et al., in rejecting claim 59 based on Ogata et al. In paragraphs 34 and 38 of the Office Action, the Examiner alleges that Goto et al. discloses it is possible to generate all possible paths for all compounds, pointing to page 596 of Goto et al. in doing so. The purpose of these components of KEGG is to delineate natural pathways of interconnected enzymes, one example of which is the well-known Krebs Cycle. In such a cyclic pathway, one chemical compound interacts with both the preceding (as product) and succeeding enzyme (as substrate) or target in the pathway. Eventually, the cycle is completed by the "last" product in the pathway acting as the substrate for the "first" enzyme in the pathway. In this regard, it does represent an interaction between one chemical and each of a plurality (two) of targets. It does not, however, represent the interaction of _each of a plurality_ of compounds in the pathway (substrates/products) and _each of a plurality_ of targets (enzymes) in the pathway. Nor would one skilled in the art find it obvious to do so from Ogata et al., because the purpose of KEGG and associated databases in Ogata et al. is to elucidate natural pathways which by the nature of the

term pathway indicates linear, or at best branched, sequences of interacting molecules, not testing the full set of interactions of each compound and each target.

Accordingly, Ogata et al. does not teach or suggest "a third database containing records corresponding to the results of tests to determine the interaction between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database."

Antman et al. is not sufficient to overcome the deficiencies of Ogata et al. for reasons similar to those provided above with respect to Goto et al. in section A. Moreover, for reasons similar to those provided above with respect to Goto et al. in section A, there is no suggestion or motivation to combine or otherwise modify Ogata et al. and Antman et al. in a way that shows the claimed invention.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claim 59 is patentable over Ogata et al. taken with Bult et al. in combination with Antman et al.

Dependent claims 10, 17, 67, 68, 79, 81-86, 92, 108, 122, and 123 are allowable not only for the reasons stated above with regard to their respective allowable base claims, but also for their own additional features that distinguish them from Ogata et al., Bult et al., and Antman et al.

Based on the above remarks, Applicants request that the Examiner withdraw this rejection.

C. **Goto et al. taken with Bult et al. in combination with Antman et al. and Wintzmann et al.**

The Examiner rejects claims 1, 17, 59, 96, 99, and 107 under 35 U.S.C. § 103(a) as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Wintzmann et al. Office Action, paragraph 75. Applicants traverse the rejection.

As noted above, Bult et al. is not prior art with respect to the present application. Accordingly, only Goto et al., Antman et al., and Wintzmann et al. remain as applicable references with respect to claims 1, 17, 59, 96, 99, and 107.

Applicants note that Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. However, none of independent claims 1 and 59, which have each been rejected as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Wintzmann et al., include this feature. Applicants request clarification on how Antman et al. applies to claims 1 and 59.

Applicants also request clarification on how Wintzmann et al. applies to claims 1 and 59. Wintzmann et al. appears to be used by the Examiner solely to attempt to show a first database comprising 2-D topological descriptors or LD50 data. Office Action, paragraphs 77-80. However, none of independent claims 1 and 59, which have each been rejected as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Wintzmann et al., include this feature.

Applicants submit that Goto et al. and Antman et al. do not disclose the features of claims 1 or 59 as explained above with reference to section A. Wintzmann et al. is not sufficient to overcome the deficiencies of Goto et al. and Antman et al. More

particularly, Wintzmann et al. does not disclose or suggest "a third database containing records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target," as recited in claim 1. Nor does Wintzmann et al. disclose or suggest "a third database containing records corresponding to the results of tests to determine the interaction between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database," as recited in claim 59.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claims 1 and 59 are patentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Wintzmann et al.

Dependent claims 17, 96, 99, and 107 are allowable not only for the reasons stated above with regard to their respective allowable base claims, but also for their own additional features that distinguish them from Goto et al., Bult et al., Antman et al., and Wintzmann et al.

Based on the above remarks, Applicants request that the Examiner withdraw this rejection.

D.   Goto et al. taken with Bult et al. in combination with Antman et al. and Schena et al.

The Examiner rejects claims 1, 17, 59, and 126 under 35 U.S.C. § 103(a) as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Schena et al. Office Action, paragraph 81. Applicants traverse the rejection.

As noted above, Bult et al. is not prior art with respect to the present application. Accordingly, only Goto et al., Antman et al., and Schena et al. remain as applicable references with respect to claims 1, 17, 59, and 126.

Applicants note that Antman et al. was only used by the Examiner to allege a teaching of a first database of chemical compounds that have failed in preclinical or human clinical tests. However, none of independent claims 1 and 59, which have each been rejected as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Schena et al., include this feature. Applicants request clarification on how Antman et al. applies to claims 1 and 59.

Applicants also request clarification on how Schena et al. applies to claims 1 and 59. Schena et al. appears to be used by the Examiner solely to attempt to show a second database with data organized by location of expression tissues. Office Action, paragraphs 83-86. However, none of independent claims 1 and 59, which have each been rejected as being unpatentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Schena et al., include this feature.

Applicants submit that Goto et al. and Antman et al. do not disclose the features of claims 1 or 59 as explained above with reference to section A. Schena et al. is not sufficient to overcome the deficiencies of Goto et al. and Antman et al. More particularly, Schena et al. does not disclose or suggest "a third database containing

records corresponding to screening results from tests of interactions between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database, the tests including information on the effect that a compound selected from the first database has on the interaction between a reference compound known to interact with a selected molecular target from the second database and said selected molecular target," as recited in claim 1. Nor does Schena et al. disclose or suggest "a third database containing records corresponding to the results of tests to determine the interaction between each of a plurality of compounds in the first database and each of a plurality of molecular targets in the second database," as recited in claim 59.

Because the references fail to teach or suggest the features that the Examiner asserts, and the Examiner provides no objective reason why one of ordinary skill in the art would have been motivated to combine the references, a *prima facie* case of obviousness has not been established. Accordingly, Applicants submit that claims 1 and 59 are patentable over Goto et al. taken with Bult et al. in combination with Antman et al. and Schena et al.

Dependent claims 17 and 126 are allowable not only for the reasons stated above with regard to their respective allowable base claims, but also for their own additional features that distinguish them from Goto et al., Bult et al., Antman et al., and Schena et al.

Based on the above remarks, Applicants request that the Examiner withdraw this rejection.

CONCLUSION

Since each of the claims is allowable, Applicants respectfully request the timely allowance of this application.
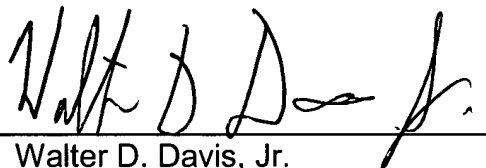
If an extension of time under 37 C.F.R. § 1.136 is required to obtain entry of this Amendment, such extension is requested. If there are any other fees due under 37 C.F.R. §§ 1.16 or 1.17 that are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: October 19, 2004   By: _____
Walter D. Davis, Jr.
Reg. No. 45,137

Attachments: Copy of U.S. Provisional Application No. 60/130,992, including Provisional Application Cover Sheet, Statement Claiming Small Entity Status, Specification, Appendices to Specification (A, B, and C).

Please type a plus sign (+) inside this box ——▶ ⊞

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

(Stamp: OIPE JC177 — OCT 1 9 2004 — PAT & TRADEMARK OFFICE)

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
David Michael	Manyak	Ellicott City, MD
Renee Ann	Zeppetello	Baltimore, MD
Hao	Chen	Adelphi, MD
Arthur David	Weissman	Baltimore, MD

[X] Additional inventors are being named on the __1__ separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

Receptor Selectivity Mapping

CORRESPONDENCE ADDRESS

Direct all correspondence to:

[ ] Customer Number _____ Type Customer Number here ——▶ Place Customer Number Bar Code Label here

OR

Firm or [X] Individual Name	Oceanix Biosciences Corporation
Address	ATTN: David Manyak
Address	7170 Standard Drive

City	Hanover	State	MD	ZIP	21076
Country	U.S.A.	Telephone	410-712-4411	Fax	410-712-4412

ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification *Number of Pages* [ ] [X] Small Entity Statement

[X] Drawing(s) *Number of Sheets* [ ] [X] Other (specify) Appendices A, B, & C

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

[X] A check or money order is enclosed to cover the filing fees

[ ] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____

FILING FEE AMOUNT ($)
$75.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE *(signature: David K. Manyak)*

TYPED or PRINTED NAME   David M. Manyak

TELEPHONE   410-712-4411 ext 106

Date   4/23/99

REGISTRATION NO. (if appropriate)   N/A

Docket Number: 99-04-26

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION COVER SHEET
Additional Page

Docket Number	99-04-26	Type a plus sign (+) inside this box → +

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Garry LeRoy	Lang	Bel Air, MD

Number __2__ of __2__

STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))—INDEPENDENT INVENTOR	Docket Number (Optional) 99-04-26

Applicant, Patentee, or Identifier: **Oceanix Biosciences Corporation**

Application or Patent No.: **Provisional Patent Application**

Filed or Issued: **April 26, 1999**

Title: **Receptor Selectivity Mapping**

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

☐ the specification filed herewith with title as listed above.

☒ the application identified above.

☐ the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☐ No such person, concern, or organization exists.

☒ Each such person, concern, or organization is listed below.
    Oceanix Biosciences Corporation
    7170 Standard Drive
    Hanover, MD 21076

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

David Manyak	Renee Zeppetello	Hao Chen
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Signature of inventor	Signature of inventor	Signature of inventor
April 23, 1999	April 23, 1999	April 23, 1999
Date	Date	Date

JIPE
OCT 1 9 2004
T & TRADEMARK OFFICE

STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))—INDEPENDENT INVENTOR	Docket Number (Optional) 99-04-26

Applicant, Patentee, or Identifier: __Oceanix Biosciences Corporation__

Application or Patent No.: __Provisional Patent Application__

Filed or Issued: __April 26, 1999__

Title: __Receptor Selectivity Mapping__

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

☐ the specification filed herewith with title as listed above.

☒ the application identified above.

☐ the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

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Oceanix Biosciences Corporation
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Hanover, MD 21076

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Arthur Weissman	Garry Lang	
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Signature of inventor	Signature of inventor	Signature of inventor
April 23, 1999	April 23, 1999	
Date	Date	Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
APPLICATION FOR A PROVISIONAL PATENT

For:

RECEPTOR SELECTIVITY MAPPING

By:

David M. Manyak
Renee A. Zeppetello
Hao Chen
Arthur D. Weissman
And
Garry L. Lang

Assigned to:

Oceanix Biosciences Corporation
7170 Standard Drive
Hanover, MD 21076

April 26, 1999

SPECIFICATION

Field of the Invention

The present invention relates to databases comprising chemical compound, molecular target, and biological/clinical information in which patterns or relationships of interactions between chemical compounds and molecular targets are determined and compared with other information in the database in order to draw conclusions that are useful for drug discovery and development and for related areas.

Background of the Invention

The worldwide pharmaceutical industry spends more than $25 billion a year on research and development, of which nearly one-third is spent on the discovery and early development phase, that period leading up to the selection of a drug candidate for preclinical and clinical development. Some critical stages in drug discovery are the following:

(1) Sequencing DNA comprising segments of the human genome;

(2) Identification of genes within the genome that are associated with specific diseases or biological functions;

(3) Production of a protein such as a receptor or enzyme that corresponds to, or is encoded by, the functional gene and which then becomes a biological or molecular target for drug discovery;

(4) Screening a library of chemical compounds for activity against the molecular target (high throughput screening);

(5) Screening the most potent active compounds against other biological targets (particularly other receptors or enzymes) to assess the compounds' selectivity or specificity for the intended biological/ molecular target and potential to cause undesirable side effects through activity at other targets;

(6) Evaluating the most potent and selective compounds for their activity in a range of other assays designed to measure such properties as toxicity, absorption, distribution, metabolism, excretion, etc.

(7) Assessing the most promising compounds based on empirical judgements using the above information, then sending that information to a chemical synthesis group to produce analogs (or modified but related chemical structures) of the initial active compounds;

(8) Retesting the chemical analogs through Steps (4), (5) and (6), then repeating Step (7) until an optimized lead compound or series of compounds is identified;

(9) Forwarding the optimized lead compounds to further preclinical and clinical testing.

Throughout this process of discovery and development, compounds go through successively narrower filters, and compounds are eventually selected for the more expensive phases of preclinical and clinical development. Unfortunately, the selection process often leads to preclinical testing and clinical testing of compounds that will fail at these stages and never reach commercialization. These failures lead to extremely high average costs, estimated to exceed $300 million, to develop and launch a new drug. If, however, the optimal drug candidate is correctly identified early in the discovery and development process and successfully passes preclinical and clinical testing, the actual cost to develop that drug may

be reduced by as much as 75%. Clearly, a major goal of pharmaceutical R&D should be to enhance the predictability of early drug development tests such as outlined above.

With the revolution of new techniques of biotechnology and the evolution of tools to automate many laboratory processes, two dominant trends have emerged in recent years that are having an important impact on pharmaceutical R&D. First, the number of molecular targets (such as new receptors and enzymes) available for discovery screening programs continues to increase dramatically due to progress in sequencing the human genome. About 200-300 molecular targets have been explored for drug discovery; estimates of the number of potential molecular targets from the human genome project range in the thousands. Second, the size of chemical compound libraries available for discovery screening programs has expanded nearly ten-fold (to more than a million compounds in many drug companies) due to automation and new technologies such as combinatorial chemistry. These two factors hold tremendous promise for new drug discovery, but they also create significant potential problems having adverse consequences on the cost of drug development. More targets and more compounds will result in many more bioactive compounds being discovered, leading to greater difficulty in selecting the optimal drug candidates to advance to preclinical testing, as well as increased development costs due to more compounds entering preclinical and clinical testing and potentially more failures at these stages.

These factors point to an increased need for rapid, inexpensive, *in vitro* assays for lead compound selection, optimization, and validation. Such rapid assays may help identify the most promising of these active compounds before they enter the later more expensive stages of drug development. These factors further point to a need for more effective methods to manage and interpret the vast amount of data on genes and gene products (molecular targets), chemical structures, and screening results.

One form of *in vitro* assay that is gaining increased importance in pharmaceutical R&D is Profiling. The NovaScreen Division of Oceanix Biosciences Corp., the Assignee for this patent application, pioneered the concept of Profiling in the late 1980's. NovaScreen provides, as a service to drug companies, an extraordinarily broad array of *in vitro* ("test-tube") assays for characterizing the pharmaceutical activity and the potential side effects of compounds under development as new drugs. NovaScreen currently performs on a routine basis more than 160 different assays (See Appendix A) based on molecular targets, called receptors and enzymes, that play a key role in a wide range of human diseases, including those associated with central nervous system disorders, immune diseases, pain and inflammation, infectious diseases, cancer, metabolism or growth factors, cardiovascular function, and the endocrine system. Pharmaceuticals accounting for more than one-half of the worldwide market function by interacting with cellular receptors. Many side effects of pharmaceuticals are also mediated through their interactions with receptors or enzymes.

Through NovaScreen's services called *PROFILE*, clients' lead compounds, generally those entering preclinical development, are tested in a battery of receptor and enzyme assays. NovaScreen's standard Side Effects *PROFILE*, for example, has 63 such assays. Information from *PROFILE* about interactions between the client's compound and certain receptors are important for the process of lead compound optimization and selection and can suggest possible side effects or secondary therapeutic activities of the compound. This knowledge can potentially save the drug company millions of dollars in wasted time and expense during preclinical and/or clinical development of the compound.

While NovaScreen's *PROFILE* services have been practiced for many years, the data generated from these tests are generally used empirically by drug companies. Most drugs, even highly selective drugs, interact with numerous receptors or other molecular targets. Interpreting *PROFILE* data, therefore, depends on the experience and knowledge of the scientist from the drug company who reviews the data

on both the chemical structure of the compounds and the binding interactions of the compounds with specific receptors. Unfortunately, even the most experienced pharmacologist has an incomplete knowledge of the interaction of different drug compounds with the broad range of receptors relevant to drug development. Scientists at NovaScreen, while having reviewed a significant amount of receptor binding data, do not know the identity of the compounds being tested due to client confidentiality and therefore have the same or greater limitations as drug company scientists with respect to interpreting full *PROFILE* data on drug candidates. Therefore, while *PROFILE* remains a very valuable tool, its predictive ability to aid new drug development has significant limitations.

The need for more effective methods to manage, collate, interpret, and utilize the vast amount of data on genes and gene products (molecular targets), chemical structures, and screening results has led to the creation of new business opportunities in bioinformatics, or managing and selling biological and chemical data. The stages of generating large pools of information for drug discovery can be broken down into the following groups:

(1) DNA sequences (code of genetic material or genes that are blueprints for the cell to make gene products or proteins);

(2) functional genomics (process of conversion of DNA sequences to expression of corresponding gene products or proteins via mRNA production, especially in response to drugs or changes in biological function);

(3) proteomics (identification of the amino acid sequence and/or three-dimensional structure of gene products or proteins, such as receptors, for which the genes code);

(4) small molecule pharmacology/toxicology (molecular binding or interactions between gene products, like receptors, and small organic chemicals that are potential drugs); and

(5) chemical structure (of small molecule, drug-like compounds).

Databases for DNA sequences (Group 1) are well established and include GenBank, The Genome Center, and others. Similarly, databases of chemical structures (Group 5) are well known and provided by vendors such as MDL (Isis) and Oxford Molecular. Databases for proteomics (Group 3), such as SWISS-PROT, ProLink, and PDB, are also being established. Each of these databases can be considered one-component, in that they contain structural information and can be used to determine patterns in that one dimension or single component of structural or sequence information. Databases for Groups 2 and 4 are not well established but should be valuable additions to the information pool for drug discovery and development. These latter two forms of datasets would be two-component or two-dimensional in that they would contain data relating to the interaction between two structures, such as genes to proteins (Group 2) and proteins to chemicals (Group 4). Such relationship databases add a significant level of complexity compared with the one-component databases.

Partial databases or datasets for Group 4 relationships have been or are being established. For example, Profiles of the binding of single compounds against a broad set of receptor targets by NovaScreen for its clients, as described above, is a partial dataset for Group 4-type databases. Similarly, data generated through high throughput screening projects in which thousands to hundreds of thousands of chemicals, such as might be contained in a chemical structure database (Group 5), are screened for activity against a specific receptor target (a single point in a Group 3 database), would represent a partial Group 4

database. Both of these partial datasets are well known as being under development by numerous entities, including many major drug companies.

Although such partial Group 4 datasets will be helpful aids for drug discovery and development, they suffer from two major drawbacks. First, they are directed toward specific two-component analyses, such as the binding selectivity of a single compound or limited set of compounds across a range of receptors (Profile) or of many compounds at one receptor target (high throughput screening). In both cases, the breadth of the dataset is insufficient to allow statistical correlations to be drawn among a multiplicity of receptor targets and a multiplicity of chemical structures. Second, and importantly, these partial datasets are being generated on chemical compounds selected for their structural novelty and therefore proprietary potential as new drugs. Since these are novel compounds, there does not exist any biological information about the activity of these compounds in humans. Such approaches therefore suffer the same limitations as the pharmacologist trying to empirically interpret the data of a Profile, as described above for NovaScreen clients in general.

SUMMARY OF THE INVENTION

The present invention relates to the novel design, construction, and application of a three-component database relating information-rich chemicals, molecular targets especially proteins or other macromolecules, and biological activity of the chemicals. Furthermore, the present invention relates to the primary use of known drugs and drug candidates that have failed in clinical or preclinical trials as a source of the chemical library for the database, together with clinical data generated for such chemicals describing their side effects, mechanism of action and other medically relevant data. The present invention further relates to determining the binding or other interactions between the chemicals and the molecular targets in the database, then using methods of relationship analysis and data mining to correlate patterns of interactions associated with specific biological activity that is relevant to drug discovery and development.

Brief Description of the Drawings

Descriptions of the drawings are attached in captions thereon.

Detailed Description

These arguments above with respect to background of the invention suggest that, contrary to standard operating procedures in the pharmaceutical industry, a Group 4 database should be established that is three-component, rather than two-component, and that it should cover a substantial breadth of both receptor or enzyme targets and chemical compounds. The third component for such a database would be created by selecting a broad set of chemical compounds that are rich in information of direct relevance to drug discovery and development. The most relevant information is obtained by actual experience of testing such chemical compounds in humans through clinical trials and/or post-marketing surveillance. Other relevant biological information comes from natural products that demonstrate one or more observed bioactivities, as well as chemical reference standards that have been used in the industry to characterize the biology of receptors. Accordingly, the preferred embodiment of information-rich chemical compounds selected for such a Group 4 database should be marketed pharmaceuticals, drugs

that have failed in clinical or preclinical trials, bioactive natural products or natural extracts, and reference agents used for receptor binding assays.

One might wish to attempt to construct such a database using screening data obtained from the scientific literature. While this approach could yield partial datasets, it would have two significant limitations. First, literature references generally provide only positive information (that is, reports of inhibition of binding of a specific compound to a specific receptor) and not negative data (i.e., a lack of inhibition of binding and therefore lack of activity). In determining useful comparisons of information, negatve data can be as valuable as positive data. Second, separate quantitative reports of binding data for one compound against a receptor in one article vs. reports of binding data for a second compound at the same receptor may not be comparable because of variations in the way the assays were performed. Therefore, the preferred embodiment for creation of a Group 4 three-component database would be to screen a broad array of compounds through a broad array of receptor or enzyme targets in order to obtain consistent comparative results and ensure the collection of negative data.

A detailed description of the invention is included in the set of presentation materials contained on the following pages (**double sided**) and elsewhere in this application.

Utility of the Receptor Selectivity Mapping Database

<u>Potential Uses of NOVASCREEN Receptor Selectivity Mapping</u>

1) Predicting the bimolecular (binding, multimolecular) interactions of an unknown compound (ligand, drug, chemical, substrate) in several biological systems (man, animal, bacteria)
2) Predicting the chemical structure (physical properties) of an unknown compound
3) Predicting the biological (therapeutic, toxicological, side effects, behavioral activity of an unknown compound
4) Predicting properties of an unknown biological entity (genomic sequence, orphan receptor, proteins, lipids, mutated known receptor) and a known biomolecular interaction.
5) Rational design of a new compound to interact with a known biological entity
6) Rational design of a new biological targets (antibodies to active sites, receptors, enzymes, carrier proteins)
7) Rational design of drug screening protocols and endpoints
8) Rational design of new chemical/drug databases (number of compounds, structure, inclusive relevant parameters, algorithms/software)
9) Rational design of automated equipment to sort, characterize, categorize or fabricate elements (receptor/ligand, enzyme/substrate. protein/protein)
10) Rational design of therapeutic approaches (antibodies, antisense DNA, protein inhibitors/stimulators, gene point mutations)

Chemistry — Screening — Pharmacology Toxicology — Clinical Trails

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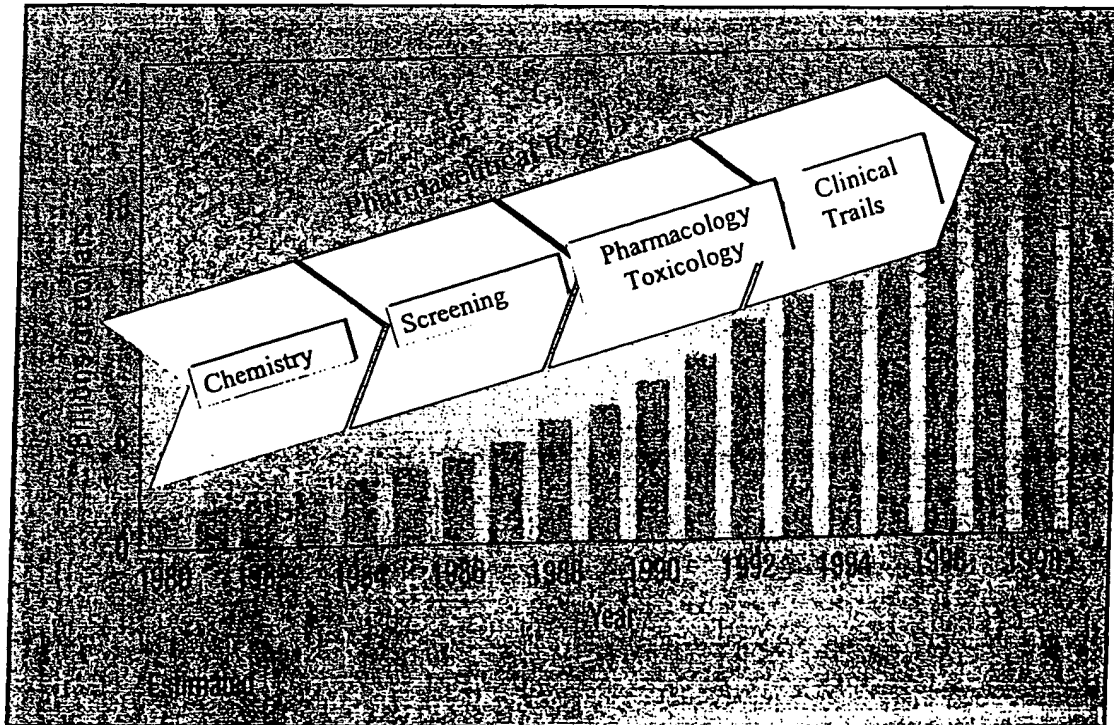
Renewed Productivity - New Drugs

Optimal Drug Candidates

Chemistry — Screening — Pharmacology — Clinical Trails

Receptor Selectivity Mapping™
    - A Multifaceted Drug Discovery & Development Database

Chemical

Receptor

Biological
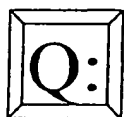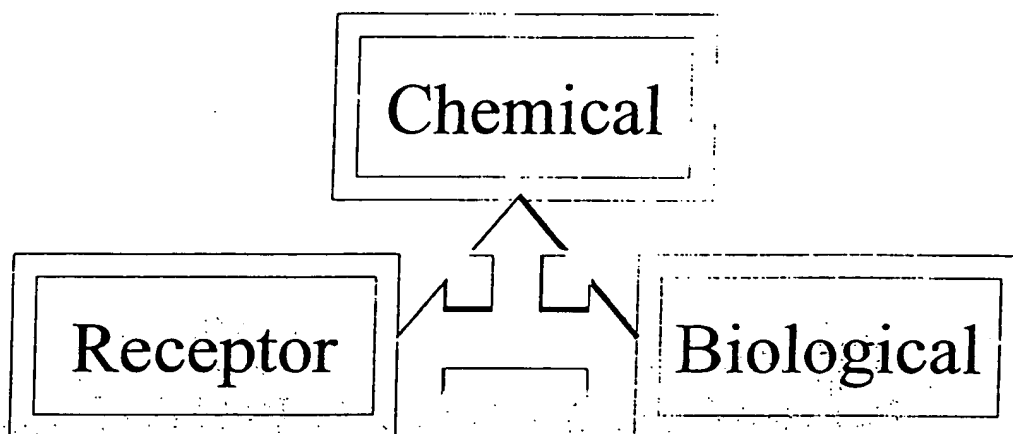
Q: Why Do You Need Such A Database?

A:
- ✓ Triage -
- ✓ Prioritization -
- ✓ Optimization -
- ✓ Discovery -

Receptor
(targets)

Classical
Drug Targets
&
Side-effect Mediators

known chemistry, biochemistry, physiology, toxicology

Convergent-
Families of different subtypes
from different animal species;
Diversified-
all inclusive list of drug targets

nature
biotechnology

Classic Dr

Selection

Adrenergic, Alpha₁, non-selective
Adrenergic, Alpha₁ₐ
Adrenergic, Alpha₁ᵦ
Adrenergic, Alpha₂, non-selective
Adrenergic, Alpha₂ₐ (human HT-29 cells)
Adrenergic, Alpha₂ₐ ₕₛ₋
Adrenergic, Alpha₂ᵦ
Adrenergic, Alpha₂ᴄ
Adrenergic, Alpha₂ᴅₐ₋
Adrenergic, Beta, non-selective
Adrenergic, Beta₁
Adrenergic, Beta₁ᵥₑₐ₋
Adrenergic, Beta₂
Adrenergic, Beta₂ᵥₑ₋
Angiotensin II, Type 1
Angiotensin II, Type 1, AT₁ᵥₑₐ₋
Angiotensin II, Type 2
Atrial Natretic Peptide, ANPₐ
Benzodiazepine (peripheral)
Bradykinin, BK₂
Bradykinin, BK₂ᵥₑₐ₋
Calcitonin Gene Related Peptide (central)
Calcitonin Gene Related Peptide (peripheral)
Calcium Channel, Type N
Calcium Channel, Type L (Dihydropyridine site)
Calcium Channel, Type L (Benzothiazepine site)
Cannabinoid, CB₁
Cannabinoid, CB₂
Cholecystokinin, CCKₐ (peripheral)

Chemical profile - 5HT3

% Inhibition (y-axis): 0, 10, 20, 30, 40, 50, 60, 70, 80, 90

Series 1

X-axis labels: +/- Baclofen, Benextramine, Benzamil, 2-BFI, BMY 7378, +/-Brmazine, BU 224, BU 239, Bupivacaine, + Butaclmol, - Butaclmol, Buspirone, Caffeine, +/-Chlorphen, Chlrprmzine, Chlrine Cl-, Cirazoline, Cortisurone, Cortisone, CPA

Serotonin, 5HT4

Chemical Profile

% Inhibitio (y-axis): 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100

X-axis labels: Substance P, LTD4, 2-Chloroadenosine (2-CADO), Phentolamine, Alprenolol, Haloperidol, R(+)-SCH-23390, Ro15-1788 (Flumazenil), Kainic Acid, Thiocoline HCl, (-)-Escopolamine, Methylβr, 8-OH-DPAT, Methysergide maleate, MDL 72222, Metoclopramide, Haloperidol, naloxone, ω-Conotoxin GVIA, (-)-MK801 Hydrogen Maleate, Apamin, Desimipramine HCl (DMI), Bradykinin TFA Salt, Neuropeptide Y (Human, ra, NBTI

Reference Compound

Chemical Profiles   5HT3 vs 5HT4

% Inhibition

Reference Compounds

S1= 5HT3;
S2=5HT4

Microsoft Access

Compound Table

Compound ID	Text
Compound	Text
CAS Number	Text
Formular	Text
Log P (pH)	Text
Hammet Sigma	Text
Other Parameters	Text
Solubility	Text
Structure	OLE Object
Smile Codes	Text
Rotatable Bonds	Text
Known Toxicology	Text
Originator	Text
Vendor	Text
Catalog Number	Text
Molecular Weight	Text
Patent	Hyperlink
Literature	Hyperlink
Comments	Memo

Chemical Snap-shot –

General Description

Name & Trade Names

Known Target(s) & Activities

Receptor Selectivity Mapping

Pharmacology & Toxicology
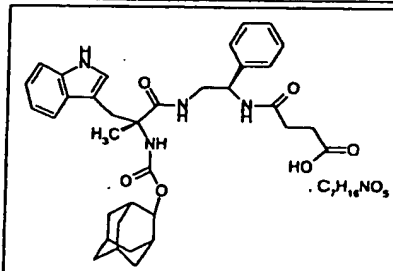
Formula : $C_{35}H_{43}N_4O_6.C_3H_7NO_3$

Octanol LogP (pH)   Hammett Sigmas

M.W.

CAS   Literature   Patent(s)

Other physical parameters: Manufacturer:

$. C_7H_{16}NO_3$

Receptor Selectivity Mapping

Alprenolol vs random selection of 55 receptors

Receptor Selectivity Mapping

Albuterol/Alprenolol vs 40 receptors

Series 1=Albuterol;
Series 2=Alprenolol

Receptor Selectivity Mapping
- A Multifaceted Drug Discovery Development Database

Chemical

Receptor

Biological

Q: <u>Who Needs This Database?</u>

A:
- ✓Drug Discovery Groups
- ✓ Lead Optimization Teams
- ✓ Toxicologists/Pharmacologiests
- ✓ Lead Selection Committees.

10,000

Compounds

- [ ] Year 4
- [ ] Year 3
- [ ] Year 2
- [ ] Year 1

Targets   250
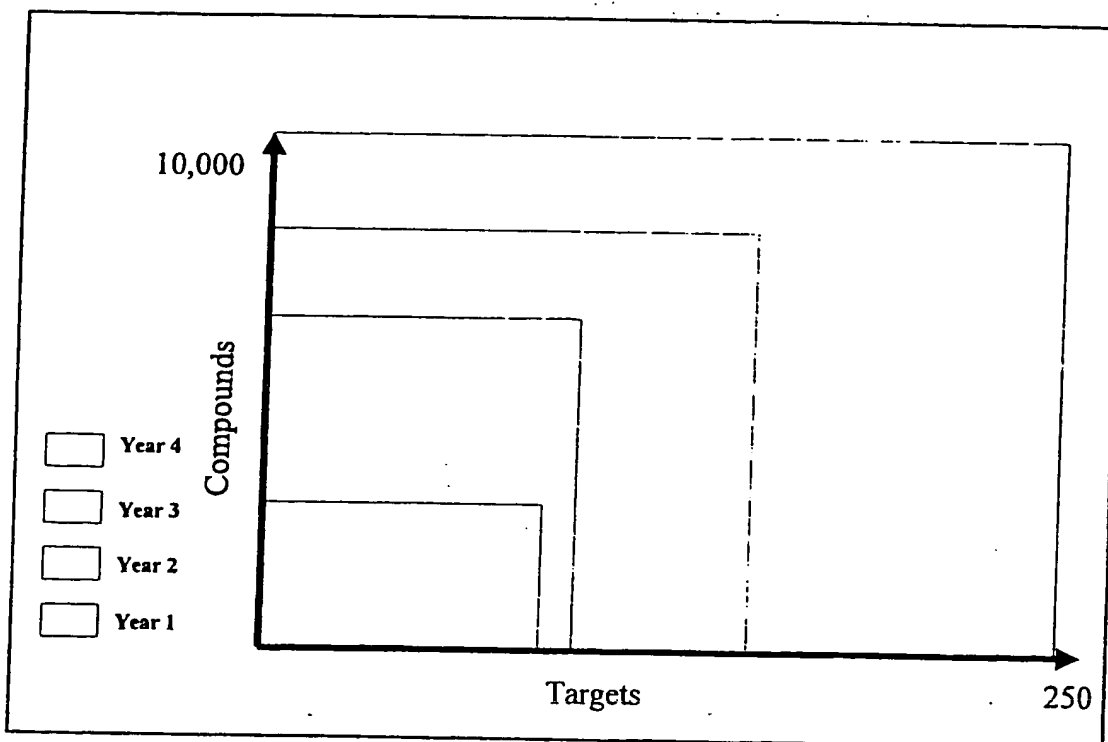
Examples

1. Selection of Chemical Libraries and Inclusion of Chemical Data

The present invention relates to databases that contain as one component chemical compounds about which information is known concerning biological activity relevant to pharmaceutical research and development. These information rich chemicals include:

(a) Compounds that are pharmacological reference agents or reference standards for measuring the interaction or molecular binding between unknown chemical compounds and a specific molecular target, such as a receptor or enzyme. Examples of such reference compounds are listed in Appendix B, which includes such compounds that are used by NovaScreen for characterizing binding interactions between test compounds and molecular targets including receptor or enzymes. Other reference agents could include chemicals selected from the catalog of Research Biochemicals Inc. (RBI), a unit of Sigma Aldrich Corp., including those chemicals listed under its LOPAC product (list also attached as Appendix B), and from other sources that are well known in the industry. These pharmacological reference compounds often have been tested previously and/or marketed as pharmaceuticals or are natural products with characterized biological activity and therefore may overlap with compounds in the following three categories.

(b) Compounds that are known pharmaceuticals that are currently or have previously been marketed for clinical use and therefore have a substantial amount of biological information available. These compounds are well-known and listed in publications available from U.S. government agencies such as the Food and Drug Administration (FDA), as well as publications by private or non-profit organizations. One such publication by a non-profit organization is the United States Pharmacopeial Convention Inc.'s *USP DI Series*, including *Volume I. Drug Information for the Health Care Professional*, which is updated monthly by *USP DI Update*. As new drugs are approved for marketing, they would be included in this category. Marketed pharmaceuticals or drugs approved by the FDA or equivalent foreign regulatory bodies are a matter of public record so that one normally skilled in the art can easily identify chemical compounds that would be included in this category.

(c) Compounds that have been approved for testing in humans, such as compounds that had been granted IND (Investigational New Drug) status, as potential drugs but that failed to achieve sufficient efficacy or safety in clinical trials to gain approval from the FDA or otherwise did not reach the status of marketed pharmaceuticals. These compounds also would have a significant amount of biological information available and would be especially useful for purposes of this invention. The identity of failed drugs can be obtained from numerous sources, including public announcements by drug and biotechnology companies, publications such as the Pink Sheets, and lists maintained by the FDA.

(d) Compounds that are obtained from natural sources such as plants, microorganisms, animals, etc., and exhibit biological activity. These natural products may include toxins, antimicrobial agents, behavioural modifiers, defensive agents, and other categories of compounds that provide information relevant to drug discovery and development. The identity of natural products can be found in numerous publications, including but not limited to the RBI catalog and Sigma Aldrich catalog of chemical compounds.

For each compound included in the database, chemical structure, chemical formulae, physical chemical characteristics, chemical space coordinates, solubility, and other relevant data, to the extent such information is available, are entered into fields in the database. Representative chemical compound parameters included in the database include those shown in the presentation materials shown above. Those skilled in the art could recognize other parameters that might be included. Chemicals can be organized by chemical structure relatedness in the database or in other relationships.

2. Selection of Receptors, Enzymes, and Other Molecular Targets and Inclusion of Molecular Target Data

Molecular targets such as receptors, enzymes, other proteins, nucleic acids, carbohydrates, and other macromolecules relevant to drug discovery and development, are the second component of the databases comprising this invention. In the preferred embodiment of this invention, receptors and enzymes are the principal molecular targets. Receptors mediate much of the molecular communication among cells and organs in the body. Enzymes often amplify such communications through, for example, secondary messenger systems and cell signaling pathways. Receptors include classical families of receptors such as dopamine receptors, serotonin receptors, opiate receptors, muscarinic receptors, adrenergic receptors, adenosine receptors, etc. These receptor groups include subtypes of the receptor type (such as dopamine-1, dopamine-2, dopamine-3, dopamine-4, and dopamine-5 receptors). Certain subtypes have further variations (such as dopamine 4.2, dopamine 4.4, and dopamine 4.7) or can have different forms (such as dopamine 2 short and dopamine 2 long). Splice variants of receptors can also occur, as can mutations in the genes encoding specific receptors which might lead to a subset of a population that has a receptor with slightly different binding affinity for drugs or other compounds compared with the normal receptor type. Receptors can be grouped by family, superfamily, or subfamily. Some groupings include G-Protein Coupled Receptors, 7 transmembrane receptors, nuclear receptors, etc. Receptors can be grouped by the degree of homology of the DNA sequence of their corresponding genes. Receptors can be grouped by their amino acid sequence and related three-dimensional conformations. Receptors can be classified by their location of expression in tissues or across different cell types.

Enzymes can include proteases, carbohydrases, kinases, phosphatases, DNA-modifying enzymes, transferases, P450's, and others listed in the Appendices and known to those skilled in the art.

A list of receptors for which assays are in routine use by the Assignee of this invention is in Appendix A. Other receptors, receptor sources, and corresponding assays are constantly being developed by the Assignee to be added to the content of the database. Additional receptors and receptor assays are well known to those skilled in the art. Lists and descriptions of certain receptors relevant to drug discovery and development can be found in numerous publications known to those skilled in the art. These publications include the RBI Handbook of Receptor Classification and the IUPHAR receptor classification book. Furthermore, as new receptors and receptor subtypes are discovered, they can be added to the content of the database.

A list of enzymes for which assays are in routine use by the Assignee of this invention is also in Appendix A and Appendix C. Other enzymes, enzyme sources, and corresponding assays are constantly being developed by the Assignee to be added to the content of the database.

Additional enzymes and enzyme assays are well known to those skilled in the art. Lists and descriptions of certain receptors relevant to drug discovery and development can be found in numerous publications known to those skilled in the art.

Representative receptor, enzyme, and other molecular target information parameters included in the database include those shown in the presentation materials above.

3. Selection of Biological/Clinical Information Parameters

Biological information to be part of the database includes material that would relate to side effects, mechanism of drug action, metabolism of a drug, toxicity, adsorption, distribution, and excretion, for example. This information is available in USP DI or on FDA-approved labels of marketed drugs, or from literature sources and publications for drugs that have failed clinical trials. Some specific parameters are listed below.

Toxicity
LD 50
LD50/ED50
Teratogenicity
Mechanism Of Toxicity
Target Organ For Toxicity
In Vitro Toxicity Battery
Induction Of Apoptosis
Bioavailability
Absorption
Blood-Brain Barrier
Oral Absorption
Mucosal Absorption
% Absorbed
Distribution
Blood Protein Bound
Half-Life
Onset Of Action
Duration Of Action
Peak Concentration In Blood
Metabolism
Major Pathway
Minor Pathway
Active Metabolites
Excretion
Primary Excretion Mode
Secondary Excretion Modes
In Vivo Effects
Therapeutic Indication
Animal Behavioral Effects
Side Effects
Primary Known Target
Other Organ/System Targets
Known Receptor Interactions

4. Establishment of PROFILE Screening Data

A key feature of this invention is the establishment of three components of information – chemicals, molecular targets, and biological – and measuring the binding interactions between the chemicals and molecular targets. This binding information can then be related back to the known biological information in order to distinguish patterns and relationships that can be used for drug discovery and development. An important aspect of this invention is to generate broad and consistent binding data between the chemicals and molecular targets in order to provide as complete a dataset as possible in order to be able to identify relevant patterns or relationships and to provide both positive and negative binding information for the datasets. In the preferred embodiment, the binding data is established as a "yes" or "no" activity for each compound and each receptor or other molecular target measured at a concentration deemed near the appropriate threshold for relevance to the biological system. For example, chemicals can be tested at 10^{-5} M (10 micromolar) for their ability to inhibit binding at a threshold of 30% between a receptor and its specific reference compound. Other initial concentrations or per cent inhibition thresholds can be selected. Also in the preferred embodiment, those chemicals that demonstrate inhibition of binding above the threshold in the initial yes/no testing are further tested for the potency of the binding inhibition. These active chemicals are tested at a series of concentrations that might, for example, include tests at 7-14 different concentrations within the range of 10^{-5} to 10^{-9} M, such that an IC-50 and/or Ki value can be determined for the active compound at the specific receptor. Fewer or more concentrations may be used for such determinations and concentrations above or below 10^{-5} to 10^{-9} M may be required. These data then yield a matrix of relative degree of activity or relative potency for each active compound at each molecular target.

In order to generate this screening data, chemicals are first solubilized in a suitable solvent system, such as 4% DMSO, although other concentrations of DMSO and other solvents are also acceptable. These chemical stock solutions are then diluted to the appropriate concentration and made available as repositories. For each assay measuring the interactions between the chemical and molecular target, the reagents and protocols for the assay will vary. Each such assay needs to be characterized and routinely established for consistency. Appropriate controls need to be run each time the assay is performed. Any assay format that can generate the desired type and accuracy of information can be used. Numerous assay detection systems, such as radioactive labels, fluorescence, chemiluminescence, UV absorption, colorimetric, etc., can be used.

In the preferred embodiment, a receptor-binding assay or enzyme activity assay is used to generate data on molecular interactions. As an example, for a receptor binding assay, chemicals from a repository are tested for their ability to inhibit the binding interaction between the receptor and a reference agent selected for that receptor. The receptor may be derived from a tissue source, such as animal or human tissue, or from a cell line expressing the receptor, or from a transfected cell line containing the gene for the receptor. The receptor source is prepared for the assays, for example by preparing a membrane fraction containing the receptor. Alternatively, the receptor may be partially purified. The reference compound, or ligand, is preferably selected for its potent and/or specific binding to the specific receptor and may have a radioactive tracer such as Iodine-125 or tritium or carbon-14 or other marker to enable bound ligand to be distinguished from unbound ligand. Coincident with testing the chemicals for binding data to include in the database, positive and negative controls are run, as is a reference curve with varying concentrations of the reference (radio)ligand to ensure the quality of the assay run. The

radioligand, receptor preparation, and test compounds are incubated together for an appropriate time, in an appropriate buffer, and at an appropriate temperature, often with the objective of reaching equilibrium of the binding reactions. The amount of bound vs. unbound radioligand is determined by a separation step such as filtration or by use of a method such as SPA (scintillation proximity assay) and measured by liquid scintillation or gamma counting. The amount of specific binding of the test compound is then determined by comparing assay results for the test chemical(s) vs. the positive and negative controls. The per cent inhibition of the test chemical(s) is calculated from these data.

NovaScreen has been engaged in the routine development and performance of such receptor binding assays for more than 10 years. NovaScreen is also routinely engaged in the development and performance of enzyme assays. Examples of assay protocols for about 150 receptor assays and enzyme assays are attached in Appendix C. Each of these may be used under this invention to generate binding interaction data between chemicals and molecular targets. One skilled in the art will recognize that numerous other receptor and enzyme assays can be performed by applying the principles described herein or from descriptions in the literature. Furthermore, one skilled in the art will recognize that other assay protocols and other detection methods, including but not limited to fluorescence assays, time-resolved fluorescence assays, fluorescence polarization assays, ELISAs, RIAs, reporter gene assays, etc., may also be used within the framework of this invention to generate binding or molecular interaction data.

Examples of datasets obtained on molecular interactions for specific receptors x a number of chemicals and for specific chemicals x a number of receptors are shown in the presentation materials above.

5. Design of Database Format and Computational Platform/Operating System

The database is constructed based on standard industry formats that are in general industry use, such as Accel, FoxPro, Oracle, Excel. Database construction is outlined in the presentation materials shown above and in the Charts to follow this page.

6. Flowcharts of Database Relationships, Queries, and Data Mining Procedures

The methods of determining relationships and analysing data are shown in the charts to follow this page.
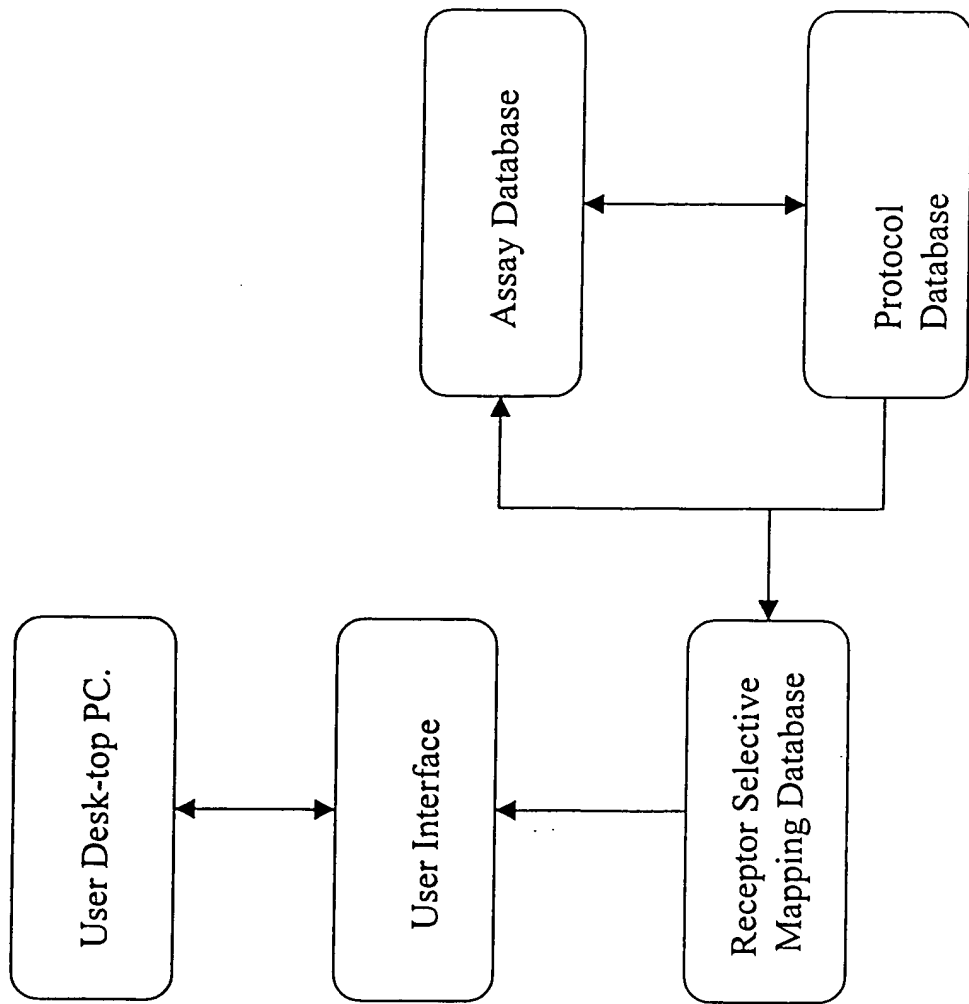
Database and user interface

Chart 1 the flow of information chart

Three-component Database For Drug Discovery/Development

```
┌─────────────────────┐              ┌─────────────────────┐
│                     │              │                     │
│ Biological target   │ ───────────► │    Receptor Selective │ ◄─────── │ Biological Database │
│ Database            │              │    Mapping Database  │
│                     │              │                     │
└─────────────────────┘              └─────────────────────┘
```

Biological target Database

Receptor Selective Mapping Database

Biological Database

Bioactive Chemical and Known Drug Database
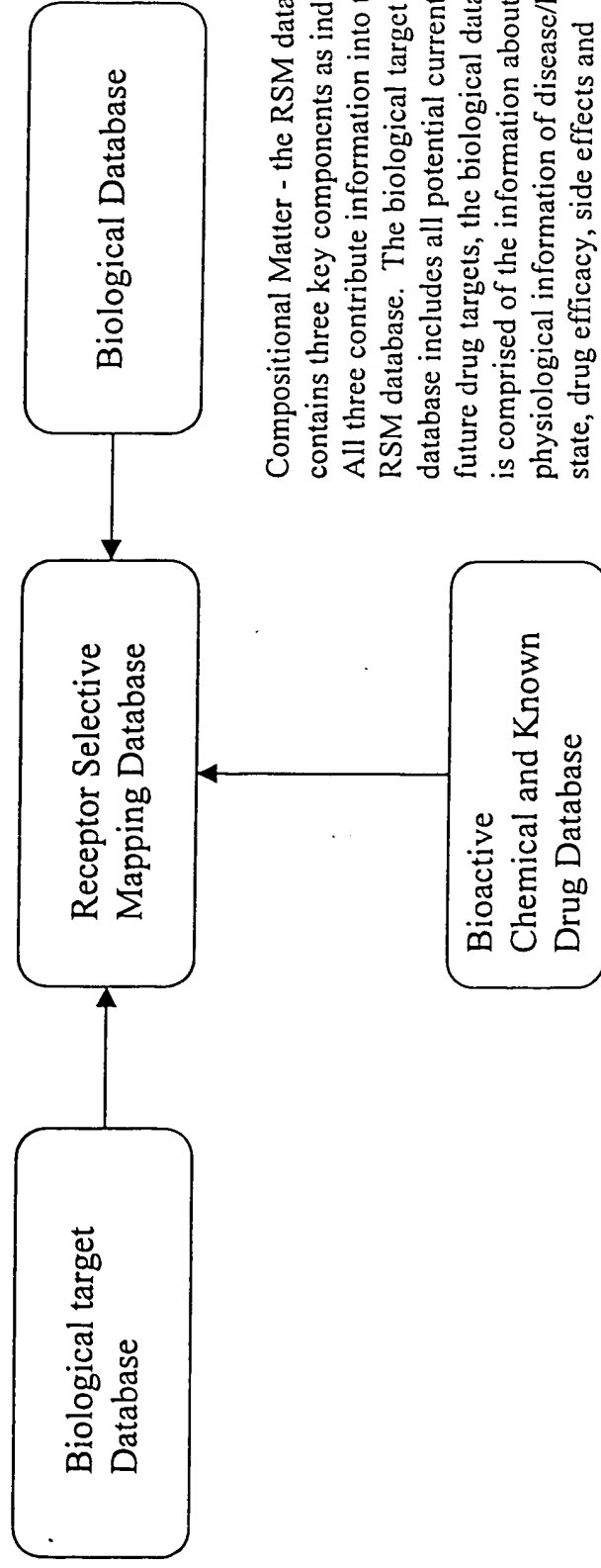
Compositional Matter - the RSM database contains three key components as indicated. All three contribute information into the RSM database. The biological target database includes all potential current and future drug targets, the biological database is comprised of the information about all physiological information of disease/healthy state, drug efficacy, side effects and toxicology. The bioactive chemical and known drug database contains chemicals that are know to be active against certain biological targets; useful in treating diseases. The chemical database also includes natural products in the form of biomass, extracts, or compounds that are not entirely from chemical synthetic origin.

Chart 2

Integration of Assay into Three-Component Database For Drug Discovery/Development
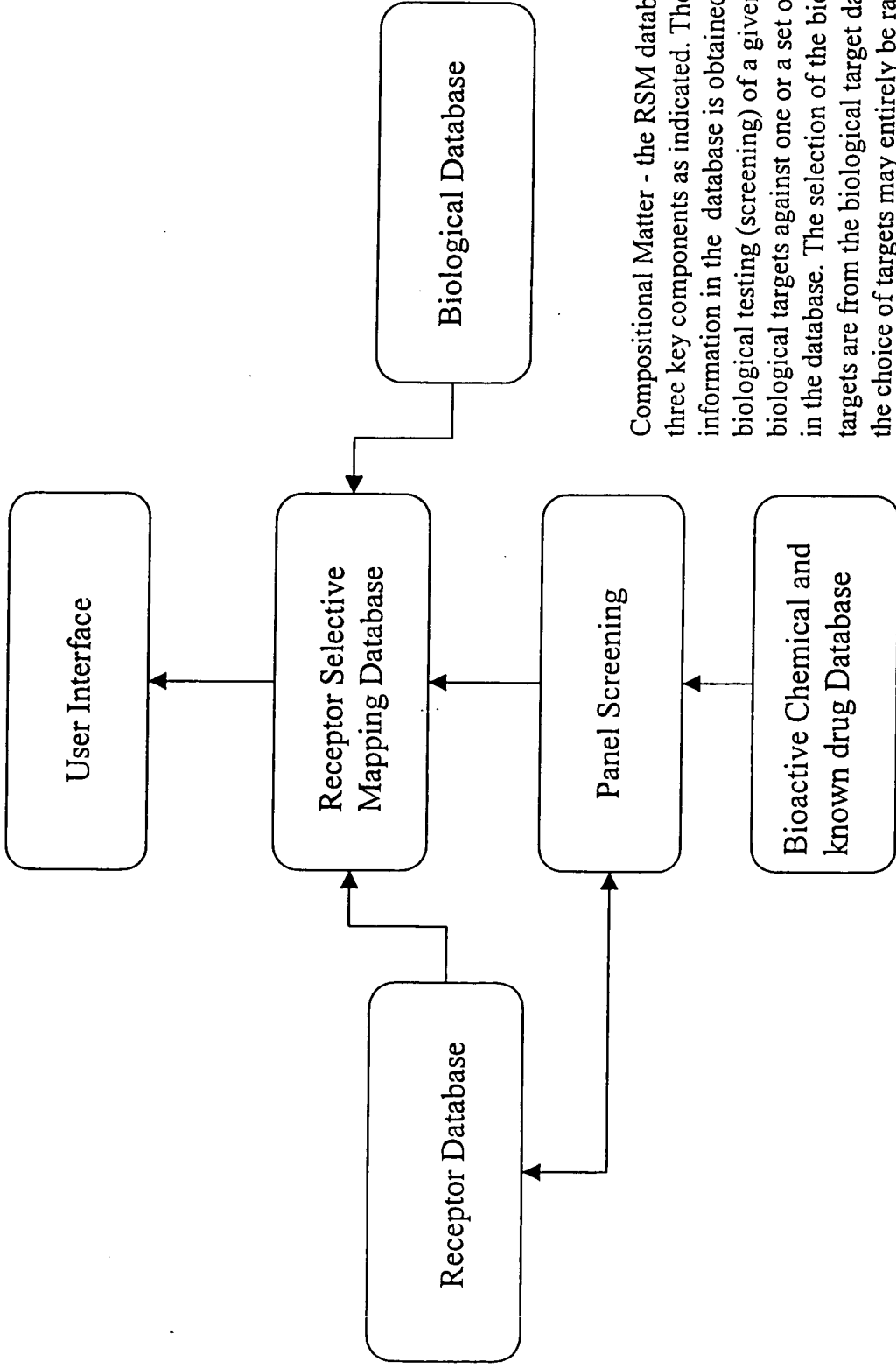
Biological Database

User Interface

Receptor Selective Mapping Database

Receptor Database

Panel Screening

Bioactive Chemical and known drug Database

Compositional Matter - the RSM database contains three key components as indicated. The chemical information in the database is obtained via biological testing (screening) of a given set of biological targets against one or a set of chemicals in the database. The selection of the biological targets are from the biological target database and the choice of targets may entirely be random, that is it may or may not be related to the original biological target or targets that the compound or these compounds are known to be actively against.

Chart 3

Integration of Assay into Three-part Content Database For Drug discovery and Development

Compositional Matter - the RSM database contains three key components as indicated. The chemical information in the database is obtained via biological testing (screening) of a given set of biological targets against one or a set of chemicals in the database. The selection of the biological targets are from the biological database and the choice of targets may entirely be random, that is it may or may not be related to that the compound or these compounds are known to be actively against

Biological Database

User Interface

Receptor Selective Mapping Database

Panel Screening

Bioactives and Known Drug Database

Receptor Database
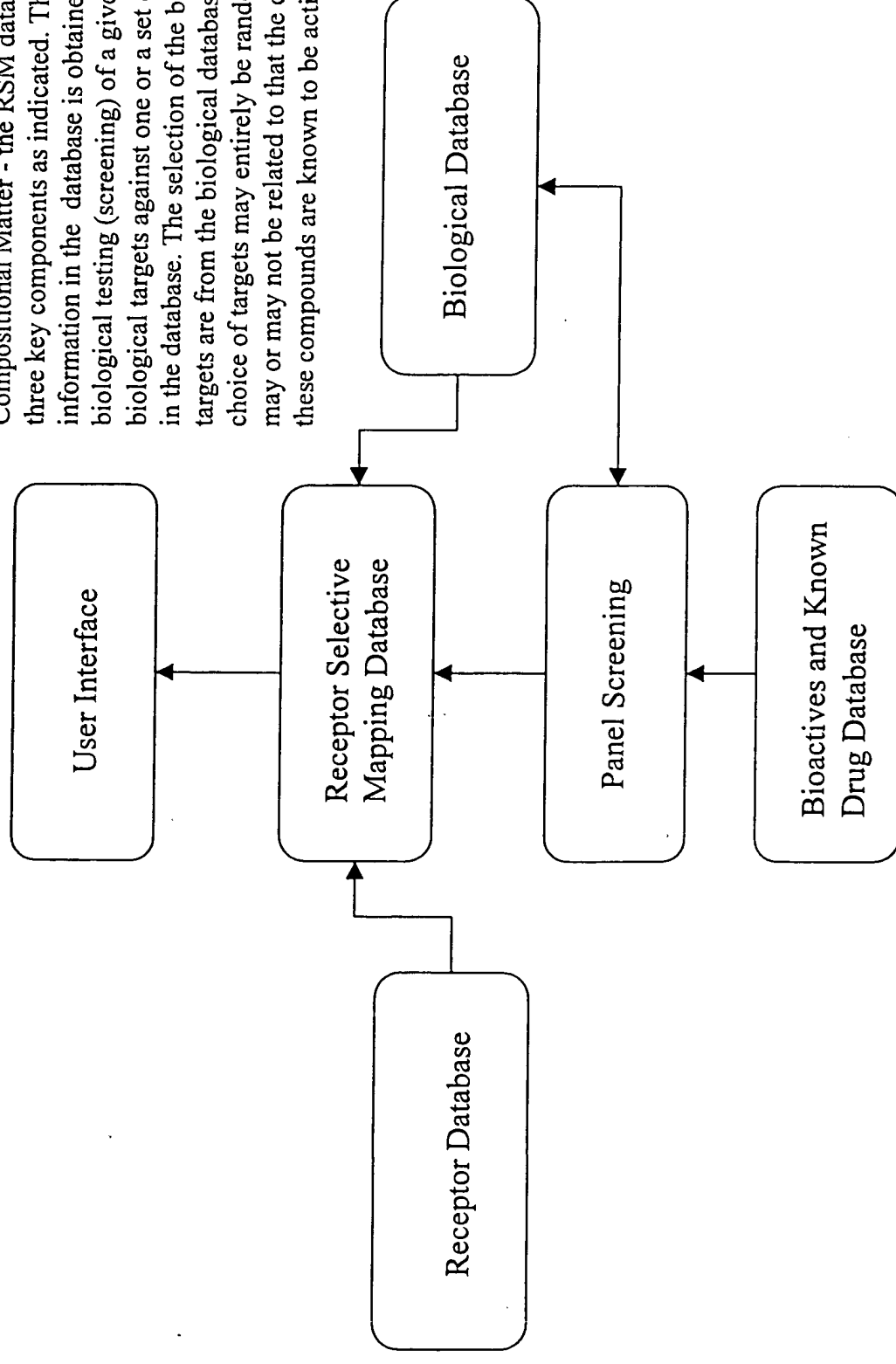
Chart 4

Integration of Assay into Three-part Content Database For Drug discovery and Development

Compositional Matter - the database contains three key components as indicated. The chemical information in the database is obtained via biological testing (screening) of a given set of biological targets against one or a set of chemicals in the database. The selection of the panel are from both target and the biological database and the choice of targets may entirely be random, that is it may or may not be related to that the compound or compounds are originally known to be.

Biological Database

User Interface

Receptor Selective Mapping Database

Target Panel Screening

Know Drug Chemical Database

Receptor Database
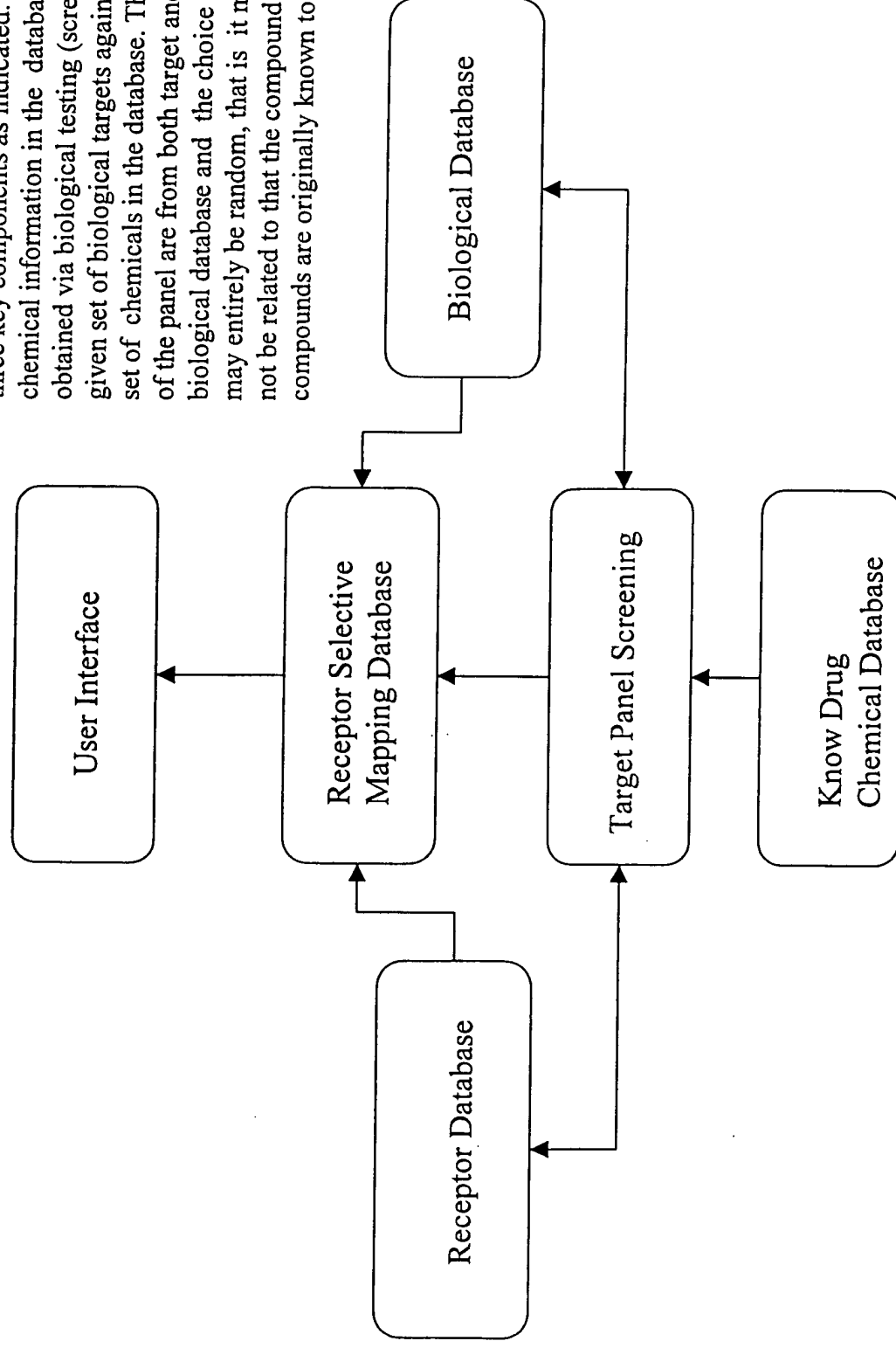
Chart 5

A seed database where other infromation maybe
added from public domain or other proprietary
data acquisition.

Receptor Selective
Mapping Database

Panel Screen

Biological Database

Known Drug
Chemical Database

Chemical Registry
Database

Receptor Database

Failed Drug Database

Genomic Database

3D structure Base

Sequence/mutation
Database

Natural product Database
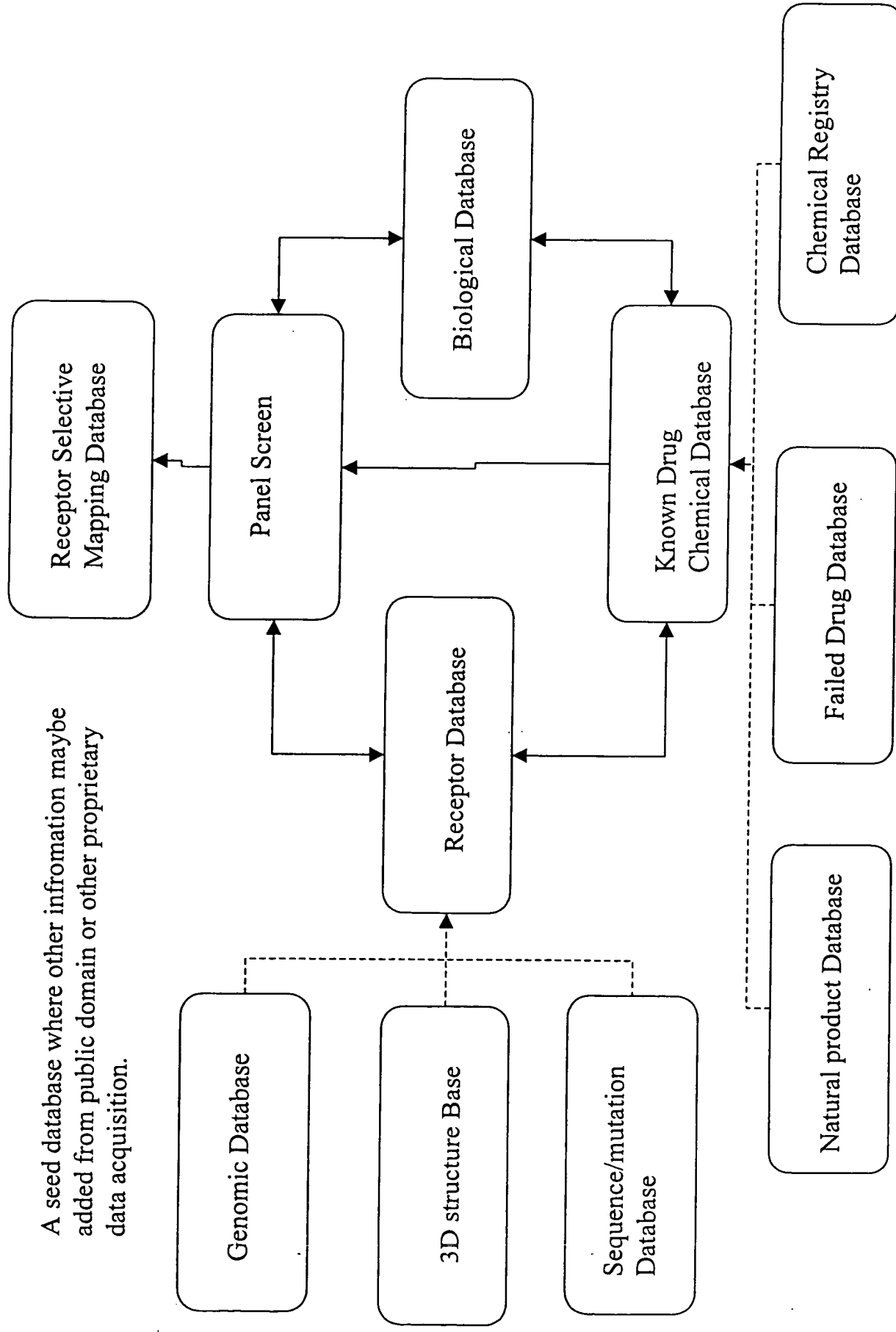
Chart 7

This and any of the prior and subsequent charts gives examples that the flow and accumulation of the information into the RSM database maybe arbitrary.


```
┌──────────────────┐
│   Biological     │
│    Database      │
└────────┬─────────┘
         │
         ▼
┌──────────────────┐         ┌──────────────────┐
│    Receptor      │◄────────│   Known Drug     │
│    Selective     │         │ Chemical Database│
│ Mapping Database │         └──────────────────┘
└────────▲─────────┘
         │
┌──────────────────┐
│    Receptor      │◄────────────┐
│    Database      │◄──┐         │
└──────────────────┘   │         │
                       │    ┌──────────────────┐
                       │    │     Genomic      │
                       │    │    Database      │
                       │    └──────────────────┘
┌──────────────────┐   │
│    3D structure  │───┘
│       Base       │
└──────────────────┘
┌──────────────────┐
│ Sequence/mutation│
│    Database       │
└──────────────────┘
```

Chart 8

Biological Database

Receptor Selective
Mapping Database

Receptor Database

Known Drug
Chemical Database

Chemical Registry
Database

Failed Drug Database

Natural product Database

Chart 9

Biological Database

Chemical Registry Database

Receptor Selective Mapping Database

Receptor Panel Screen

Known Drug Chemical Database

Failed Drug Database

Receptor Database

Natural product Database

Chart 10

Chart 11

All mentioned publications are hereby incorporated in their entirety by reference.

While the forgoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from reading of the disclosure that various changes in form and detail can be made without departing from the true scope of the invention. Furthermore, while the descriptions have been directed toward examples and applications for drug discovery and development, it will be apparent to those skilled in the art that other applications of this invention exist. Some examples of other fields of application could include, but are not be limited to, (1) discovery and development of agrichemicals such as herbicides, pesticides, insecticides, growth-regulating compounds, etc.; (2) determination of safety and efficacy of Environmental Protection Agency regulated chemicals including those that might be released into the environment and especially compounds such as endocrine mimics; and (3) ingredients in foods and/or consumer products such as dental and other oral products, health care products, personal care products, cosmetics, or topically applied substances.

References Cited

The RBI Handbook of Receptor Classification and Signal Transduction, 3rd Edition, Kenneth J. Watling, Ph.D., Natick, MA

USP DI: Drug Informatin for the Health Care Professional. 1996 (and earlier or later editions), The U.S. Pharmacopeial Convention Inc., Rockville, MD.

RBI Catalog

Sigma Aldrich Catalog

Drawings

(See text)

APPENDICES

(Attached, following)

Table of Contents

* human or rat recombinant receptor assays
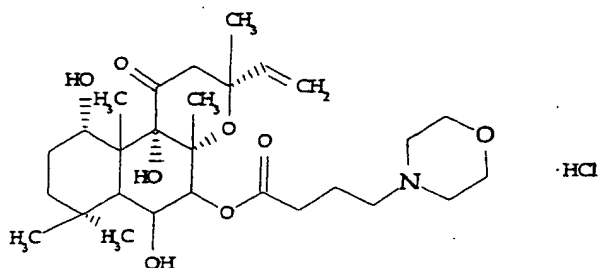**Under development

Table of Contents

5-Amino valeric acid (*5-NH$_2$-valerate hydrochloride*)
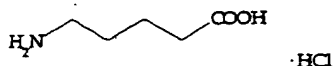
*A-120

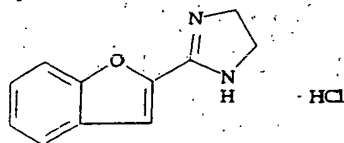Gamma-Aminobutyric Acid, GABA$_B$

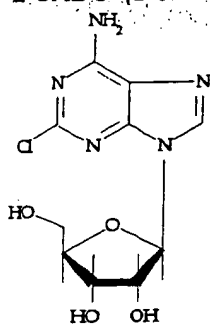### 7β-deacetyl-7-β-butyrlforskolin hydrochloride

*F-111

Adenylate Cyclase *(Forskolin)*

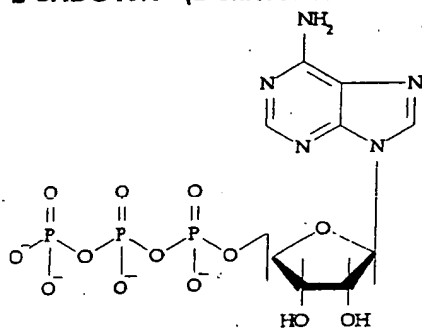### 2-BFI (*2-(2-Benzofuranyl)-2-imidozoline hydrochloride*)

Imidazoline$_2$

2-CADO (*2-Chloroadenosine*)

*A-019

Adenosine (Non-selective)
Adenosine, A$_1$
Adenosine, A$_2$

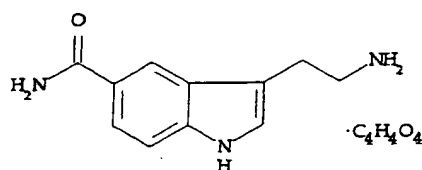### 2-CADO ATP (*2-Chloroadenosine ATP*)
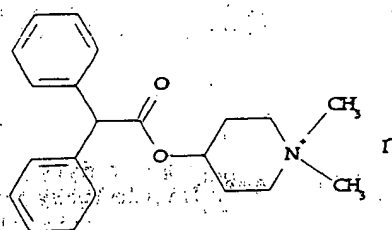

C-145

Purinergic, P$_{2Y}$

1

3-α-Chloroimperialine

Muscarinic$_1$ (Human Recombinant)
Muscarinic$_2$ (Human Recombinant)
Muscarinic$_3$ (Human Recombinant)
Muscarinic$_4$ (Human Recombinant)

## 5-CT (5-Carboxamidotryptamine maleate)

H_2N — C(=O) — [indole ring] — $CH_2CH_2NH_2$ · $C_4H_4O_4$

*C-117

Serotonin, 5HT$_1$
Serotonin, 5HT$_{1D}$
Serotonin, 5HT$_6$ (Rat Recombinant)
Serotonin, 5HT$_7$ (Rat Recombinant)

4-DAMP (4-Diphenylacetoxy-N-methylpiperidine methiodide)

*D-104

Muscarinic$_1$
Muscarinic$_2$
Muscarinic$_3$
Muscarinic$_1$ (Human Recombinant)
Muscarinic$_2$ (Human Recombinant)
Muscarinic$_3$ (Human Recombinant)
Muscarinic$_4$ (Human Recombinant)
Muscarinic$_5$ (Human Recombinant)
Muscarinic, Non-Selective (Central)
Muscarinic, Non-Selective (Peripheral)
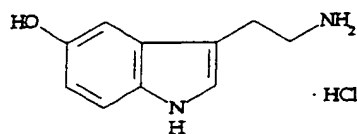
5,7 DCKA (5,7- Dichlorokynurenic acid)
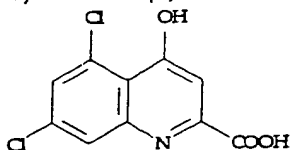
*D-138

Glycine, Strychnine-Insensitive

7-Deacetylforskolin
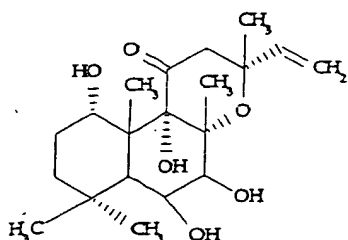
Adenylate Cyclase (Forskolin)

5-HT *(5-Hydroxytryptamine hydrochloride, Serotonin)*

*S-011

Serotonin, 5HT$_1$
Serotonin, 5HT$_{1A}$
Serotonin, 5HT$_{1B}$
Serotonin, 5HT$_{1D}$
Serotonin, 5HT$_2$
Serotonin, 5HT$_3$
Serotonin, 5HT$_4$
Serotonin, 5HT$_6$ *(Rat Recombinant)*
Serotonin, 5HT$_7$ *(Rat Recombinant)*
Serotonin, 5HT Uptake

7-O-Hemisuccinyl-7-deacetylforskolin
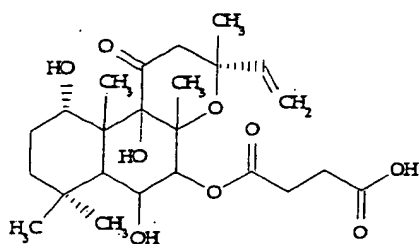
Adenylate Cyclase *(Forskolin)*

6-Hydroxymelatonin
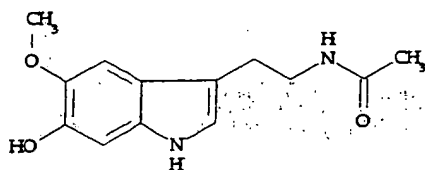
Melatonin

2-Iodomelatonin
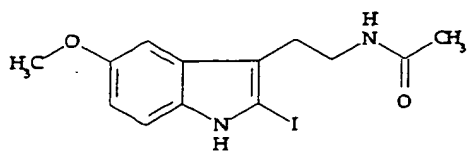
*M-112

Melatonin

5-Methoxyindole
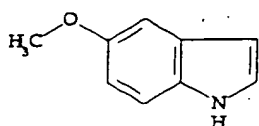
Melatonin

5-Methoxytryptamine
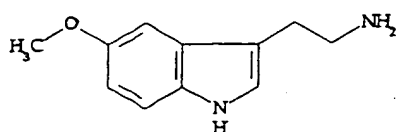
Melatonin
Serotonin, 5HT *(Non-Selective)*
Serotonin, 5HT$_1$

3

5-Methylurapidil
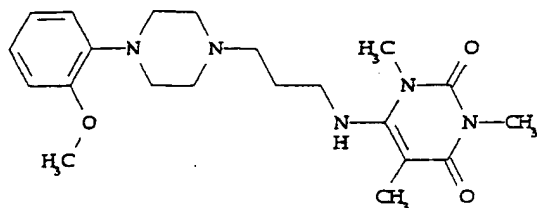
*U-101

Adrenergic, Alpha$_{1A}$
Adrenergic, Alpha$_{1B}$

5-Methoxytryptophol
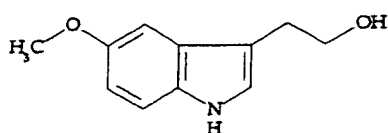
Melatonin

3-MeHis^2-TRH (3-Methyl-His^2-Thyrotropin Releasing Hormone)

Glu-3Me-His-Pro

Thyrotropin Releasing Hormone (TRH)

(±)-7-OH-DPAT (7-Hydroxy-dipropyl-aminotetralin hydrobromide)
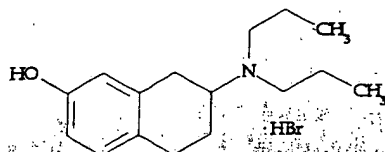
*H-145

Dopamine$_3$ (Rat Recombinant)

(±)-8-OH-DPAT (8-Hydroxy-dipropy-laminotetralin hydrobromide)
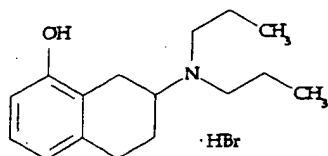
*S-002

Serotonin, 5HT$_{1A}$
Serotonin, 5HT$_{1D}$

20-OH-LTB$_4$ (20-Hydroxy-leukotriene B$_4$)
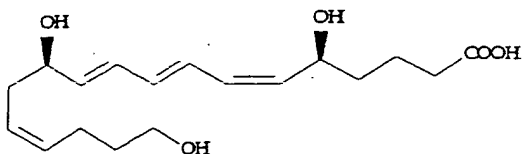
Leukotriene B$_4$ (LTB$_4$)

R(+)-3-PPP *((+)-3-(3-Hydroxyphenyl)-N-propylpiperidin hydrochloride)*

OH

·HCl

*P-102
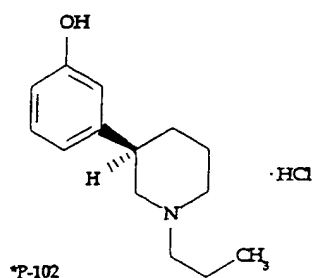
Sigma *(Non-Selective)*
Sigma$_1$
Phencyclidine *(PCP)*

Acetyl pepstatin

Ac-Val-Val-Sta-Ala-Sta

Sta= Statine

HIV-1 Protease
HIV-2 Protease

N-Acetyltryptamine
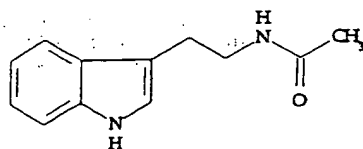
Melatonin

Aconitine
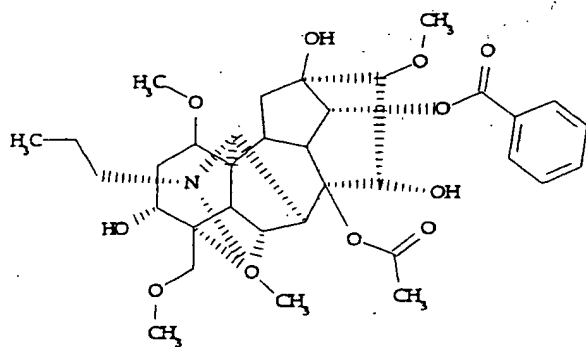
Sodium Channel, Site 2 *(Batrachotoxin)*

ADP *(Adenosine 5'-diphosphate)*
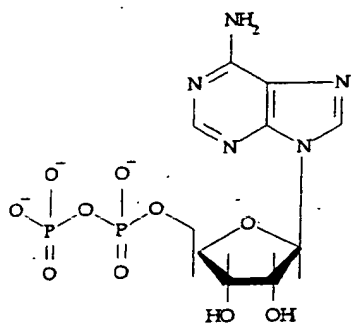
Purinergic, P$_{2Y}$

ADPβS *(Adenosine 5'-(β-thio)diphosphate)*
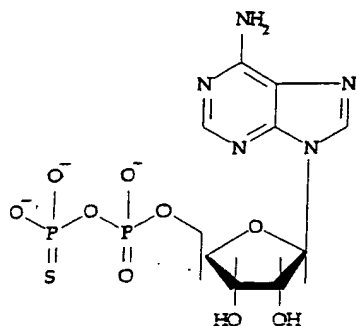
Purinergic, P_{2Y}

Agmatine sulfate

*A-198
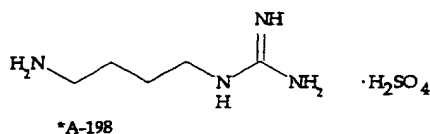
Adrenergic, Alpha$_2$ *(Non-Selective)*
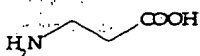
β-Alanine

Glycine, Strychnine-Insensitive

D-Alanine
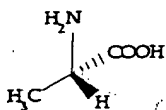
Glycine, Strychnine-Insensitive

Albuterol
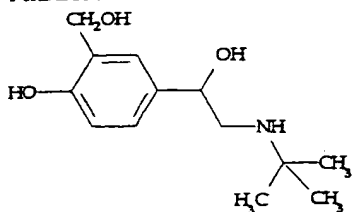
Adrenergic, Beta *(Non-Selective)*

Alprenolol hydrochloride

*A-131
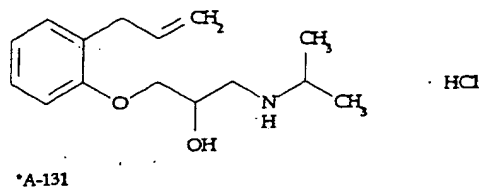
Adrenergic, Beta *(Non-Selective)*
Adrenergic, Beta$_1$
Adrenergic, Beta$_2$

L-Aminoadipic acid
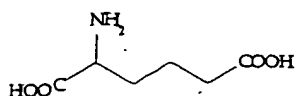
Glutamate Uptake

Aminoguanidine
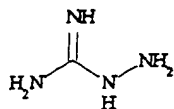
Nitric Oxide Synthetase *(NOS)*

Aminophylline ethylenediamine
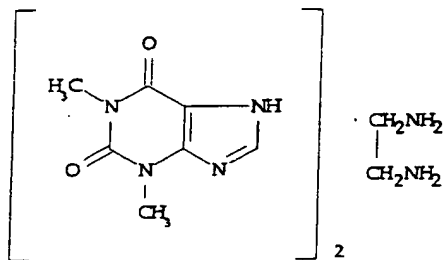
Adenosine, A$_2$

*A-107

(±) AMPA ((±) α-*Amino-3-hydroxy-5-methylisoxazole-4- propionic acid hydrobromide)*
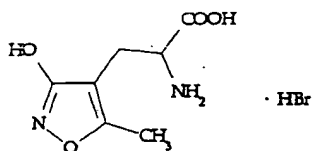
Glutamate *(Non-Selective)*
AMPA

*G-017

Angiotensin II, human

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

*A-151

Angiotensin II, Type 1 *(Central)*
Angiotensin II, Type 2 *(Peripheral)*

[Sar^1-Ile^8]-Angiotensin II

Sar-Arg-Val-Tyr-Ile-His-Pro-Ile

Angiotensin II, Type 1 *(Central)*
Angiotensin II, Type 2 *(Peripheral)*

Angiotensin I $_{(1-6)}$

Asp-Arg-Val-Tyr-Ile-His

Angiotensin II, Type 1 *(Central)*
Angiotensin II, Type 2 *(Peripheral)*

rANP *(Atrial natriuretic peptide, rat)*

Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-
Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-
Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr

Atrial Natriuretic Factor, ANF$_1$

(±) AP-4 ((±) 2-Amino-4-phosphonobutyric acid)

*A-102

N-Methyl-D-Aspartate (NMDA)
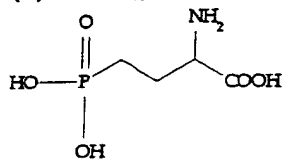
(±) AP-7 (±) 2-Amino-7-phosphonoheptanoic acid)

*G-018

N-Methyl-D-Aspartate (NMDA)
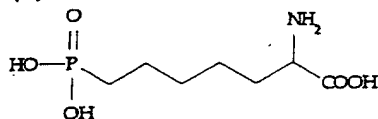
Apamin

Cys-Asn-Cys-Lys-Ala-Pro-Glu-Thr-Ala-
Leu-Cys-Ala-Arg-Arg-Cys-Gln-Gln-His

*A-146

Potassium Channel, Low Conduct. Ca^{2+} Activated
(Apamin)

Atropine sulfate

$\cdot H_2SO_4$
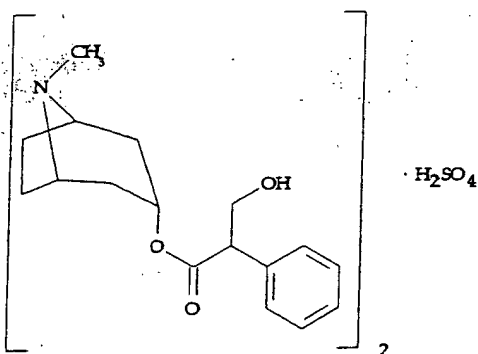
*A-105

Muscarinic, Non-Selective (Central)
Muscarinic, Non-Selective (Peripheral)
Muscarinic$_1$
Muscarinic$_2$
Muscarinic$_1$ (Human Recombinant)
Muscarinic$_2$ (Human Recombinant)
Muscarinic$_3$ (Human Recombinant)
Muscarinic$_4$ (Human Recombinant)
Muscarinic$_5$ (Human Recombinant)

(±)-Baclofen
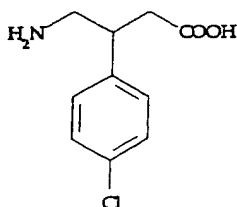
*B-020

Gamma-Aminobutyric Acid, GABA$_B$

Benzotript
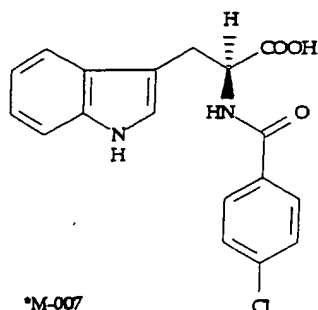
*M-007

Cholecystokinin, CCK$_A$
Cholecystokinin, CCK$_B$

BIMU

Serotonin, 5HT$_4$

BMY 7378 dihydrochloride

·2HCl
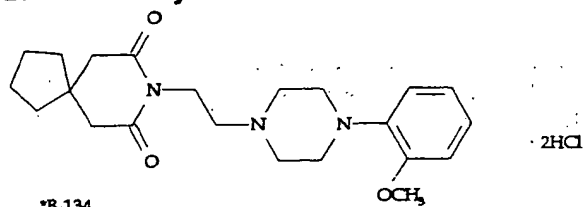
*B-134

Serotonin, 5HT$_{1A}$

Bombesin

Glu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-
Ala-Val-Gly-His-Leu-Met

Gastrin Releasing Peptide

[Tyr^4]-Bombesin

Glu-Gln-Arg-Tyr-Gly-Asn-Gln-Trp-
Ala-Val-Gly-His-Leu-Met

Gastrin Releasing Peptide

Bradykinin

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

*B-120

Bradykinin, BK$_2$

[Des-Arg^0,Hyp^3,Thi^{5,8} Des-Phe^7]-Bradykinin

D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg

Bradykinin, BK$_2$

[Des-Arg^9]-Bradykinin

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe

Bradykinin, BK$_2$

9

[Des-Arg^0,Hyp^3,Des-Phe^7]-Bradykinin

D-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-D-Phe-Phe-Arg

Bradykinin, BK$_2$

[Lys^0]-Bradykinin *(Kalladin)*

Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Bradykinin, BK$_2$

BU224 *(2-(4,5-Dihydroimidaz-2-yl) quinoline hydrochloride)*

Imidazoline$_2$

· HCl
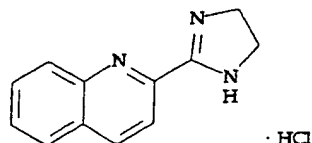
BU239 *(2-(4,5-Dihydroimidaz-2-yl) quinoxaline hydrochloride)*

Imidazoline$_2$

· HCl
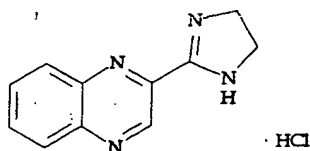
Bupivacaine
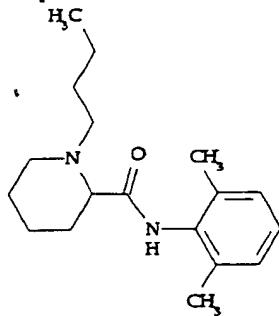
Sodium Channel, Site 2 *(Batrachotoxin)*

Bupropion hydrochloride

Dopamine Uptake

· HCl

*B-102

(+)-Butaclamol hydrochloride

OH
CH₃
CH₃
CH₃
·HCl
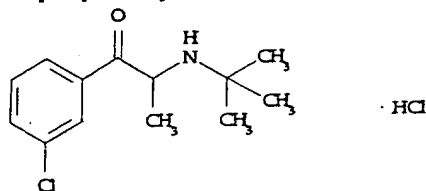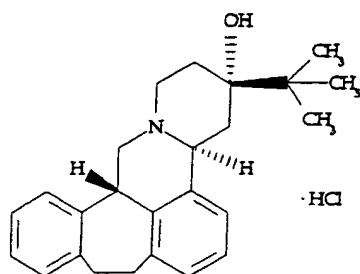
*D-033

Dopamine *(Non-Selective)*
Dopamine₁
Dopamine₂

---

rC5a *(Recombinant C5a, human)*

Complement C₅ₐ

Carbachol
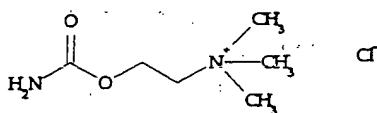
C-107

Nicotinic

CCK₈ sulfated *(Cholecystokinin ₂₆₋₃₃)*

Asp-Tyr(SO3H)-Met-Gly-Trp-Met-Asp-Phe

· 2NH3

*C-151

Cholecystokinin *(Peripheral, CCK_A)*
Cholecystokinin *(Central, CCK_B)*

CCK₈ non-sulfated *(Cholecystokinin ₂₆₋₃₃)*

Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe

*C-152

Cholecystokinin *(Peripheral, CCK_A)*
Cholecystokinin *(Central, CCK_B)*

CGS 19755

PO₃H₂
COOH
N
H
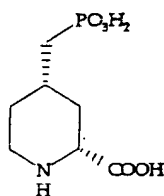
*C-105

N-Methyl-D-Aspartate *(NMDA)*

11

CGS 12066A maleate

· $C_4H_4O_4$
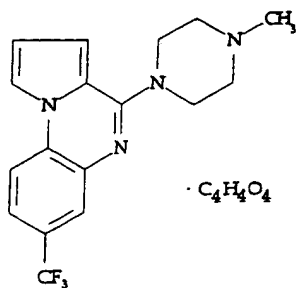
*C-106

Serotonin$_1$
Serotonin$_{1B}$

CGS 21680 hydrochloride

·HCl
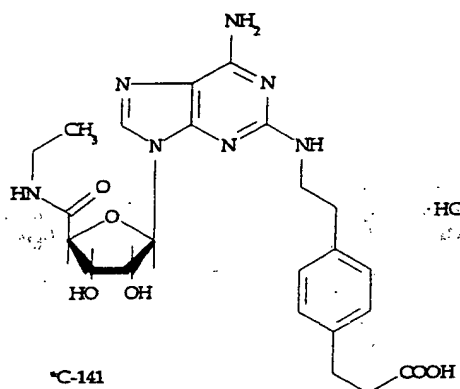
*C-141

Adenosine$_2$

hCGRP *(Calcitonin gene-related peptide, human)*

Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-
Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-
Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-
Gly-Ser-Lys-Ala-Phe

Calcitonin Gene-Related Peptide, Ty 1 *(Central)*
Calcitonin Gene-Related Peptide, Ty 2 *(Peripheral)*

hCGRPβ *(Calcitonin gene-related peptide β)*

Ala-Cys-Asn-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-
Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Met-
Val-Lys-Ser-Asn-Phe-Val-Pro-Thr-Asn-Val-
Gly-Ser-Lys-Ala-Phe

Calcitonin Gene-Related Peptide, Ty 1 *(Central)*
Calcitonin Gene-Related Peptide, Ty 2
(Peripheral)

CHA (N[6]-*Cyclohexyladenosine*)

*A-002
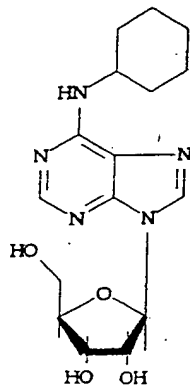
Adenosine$_1$
Adenosine$_2$

Charybdotoxin

Glu-Phe-Thr-Asn-Val-Ser-Cys-Thr-Thr-Ser-Lys-Glu-Cys-
Trp-Ser-Val-Cys-Gln-Arg-Leu-His-Asn-Thr-Ser-Arg-Gly-
Lys-Cys-Met-Asn-Lys-Lys-Cys-Arg-Cys-Try-Ser

*C-133

Potassium Channel, Voltage Dependent
(Charybdotoxin)

1-(3-Chlorophenyl)piperazine dihydrochloride

· 2HCl
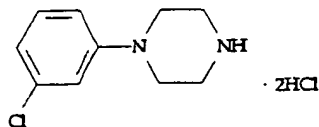
S-014

Serotonin$_{1B}$

(±)-Chlorpheniramine maleate

· C$_4$H$_4$O$_4$
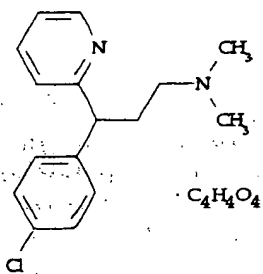
*C-119

Histamine$_1$

Choline chloride
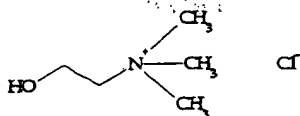
Choline Uptake

Cimetidine (SKF 92334)
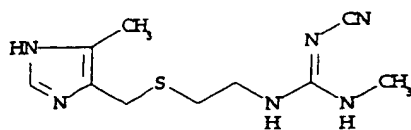
*C-118

Histamine$_2$

Clomipramine hydrochloride
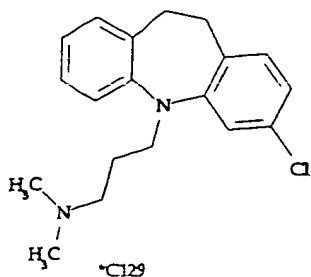
*C129

Serotonin Uptake

13

Clonazepam
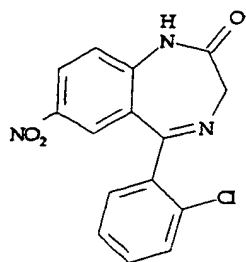
Benzodiazepine *(Central)*

Clonidine hydrochloride
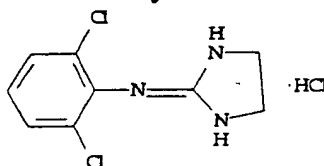
·HCl

*B-001

Adrenergic, Alpha$_2$ *(Non-Selective)*

Clorgyline hydrochloride

·HCl
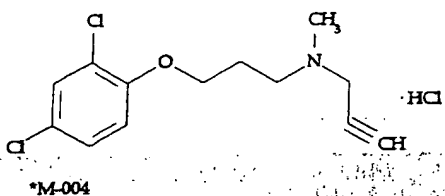
*M-004

Monoamine Oxidase *(MAO$_A$)*
Monoamine Oxidase *(MAO$_B$)*
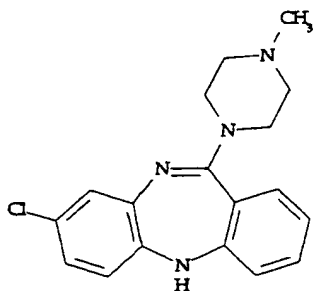
Clozapine

*C-171

Clozapine
Serotonin$_{2C}$

CNQX *(6-Cyano-7-nitroquinoxaline-2,3-dione)*
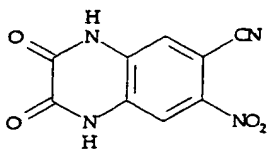
*C-127

AMPA

ω-Conotoxin GVIA

Cys-Lys-Ser-Hyp-Gly-Ser-Ser-Cys-Ser-Hyp-
Thr-Ser-Tyr-Asn-Cys-Cys-Arg-Ser-Cys-Asn-
Hyp-Tyr-Thr-Lys-Arg-Cys-Tyr

Calcium Channel, Type N *(ω-conotoxin)*

Corticosterone
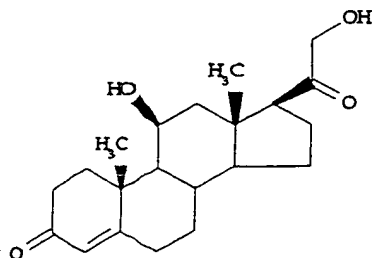
*C-173

Progesterone

CPA *(N⁶-Cyclopentyladenosine)*
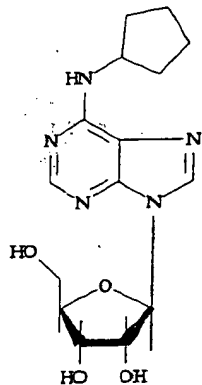
*A-017

Adenosine₁
Adenosine₂

(±) CPP (±) *3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid)*
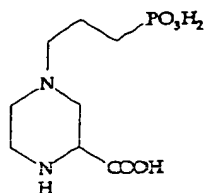
*C-104

N-Methyl-D-Aspartate *(NMDA)*

CPX (DPCPX, 8-Cyclopentyl-1,3-dipropylxanthine, PD 116,948)

Adenosine$_2$

*C-101

---
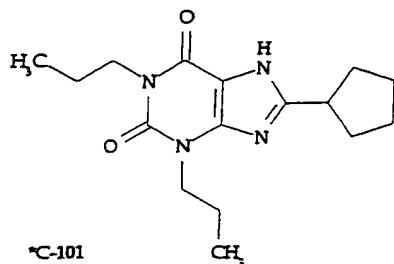
oCRF (*Corticotropin releasing factor, ovine*)

Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-
Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-
Met-Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln-
Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala

Corticotropin Releasing Factor *(CRF)*

---

tyr^0-oCRF (tyr^0- *Corticotropin releasing factor, ovine)*

Tyr-Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-
Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-
Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln-Ala-His-
Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala

Corticotropin Releasing Factor *(CRF)*

---

r/h CRF (*Corticotropin releasing factor, rat/human*)

Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-
Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-
Arg-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-
Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile

Corticotropin Releasing Factor *(CRF)*

α helical CRF antagonist (9-41)

Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-
Met-Leu-Glu-Met-Ala-Lys-Ala-Glu-Gln-Glu-
Ala-Glu-Gln-Ala-Ala-Leu-Asn-Arg-Leu-Leu-
Leu-Glu-Glu-Ala

Corticotropin Releasing Factor *(CRF)*

α helical CRF antagonist (12-41)

Phe-His-Leu-Leu-Arg-Glu-Met-Leu-Glu-Met-
Ala-Lys-Ala-Glu-Gln-Glu-Ala-Glu-Gln-Ala-
Ala-Leu-Asn-Arg-Leu-Leu-Leu-Glu-Glu-Ala

Corticotropin Releasing Factor *(CRF)*

(+)-Cyclazocine
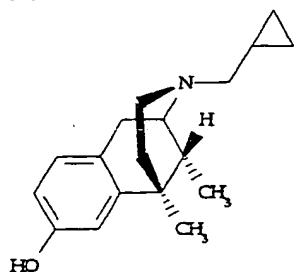
Opiate *(Non-Selective)*
Opiate, Mu *(Non-Selective)*
Opiate, Delta

*C-147

Cyproheptadine hydrochloride

· HCl
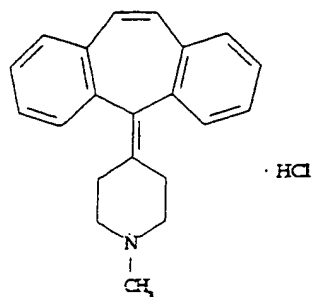
•C-112

Histamine₁ → **Histamine$_1$**

DADLE ([D-Ala², D-Leu⁵]-Enkephalin)

DADLE (*[D-Ala^2, D-Leu^5]-Enkephalin*)

Tyr-D-Ala-Gly-Phe-D-Leu

•E-116

Opiate, Delta
Opiate, Mu *(Non-selective)*

DAMGO ([D-Ala², N-Me-Phe⁴, Gly-ol⁵]-Enkephalin)

DAMGO (*[D-Ala^2, N-Me-Phe^4, Gly-ol^5]-Enkephalin*)

Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol

•D-139

Opiate, Delta
Opiate, Mu *(Non-selective)*

R(-)-Deprenyl hydrochloride
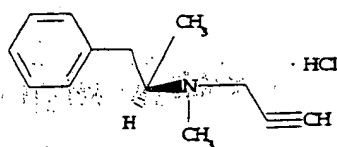
•M-003

Monoamine Oxidase *(MAO$_A$)*
Monoamine Oxidase *(MAO$_B$)*

Dexamethasone
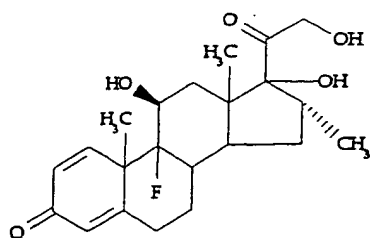
•D-157

Testosterone

S(+)-Dexetimide hydrochloride
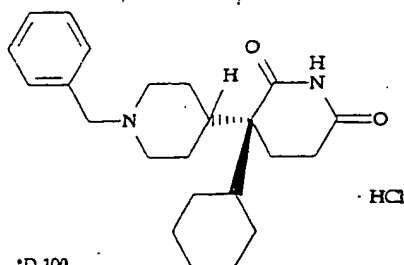
•D-100

Muscarinic, Non-Selective *(Peripheral)*

17

Diazepam *(Ro 05-2807)*
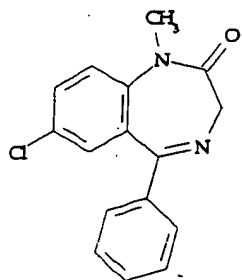
Benzodiazepine *(Central)*
Benzodiazepine *(Peripheral)*

*D-120

Dibucaine hydrochloride

Sodium Channel, Site 2 *(Batrachotoxin)*

· HCl
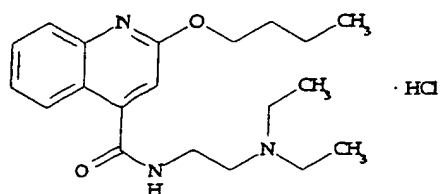
Diethylstilbestrol
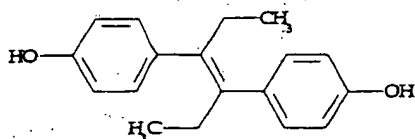
Estradiol

Dihydrotestosterone *(5-α-Androstan-17-β-ol-3-one)*
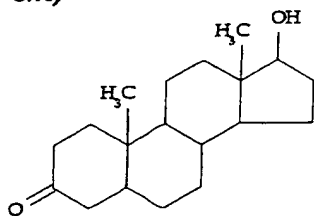
Testosterone

Dimaprit dihydrochloride

Histamine$_2$

· 2HCl
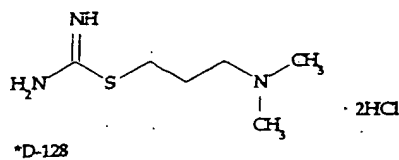
*D-128

Diphenhydramine hydrochloride
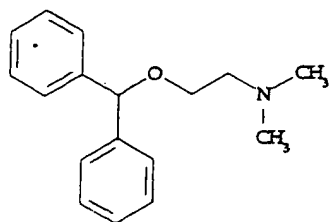
*D-158

Histamine₁

Dipyridamole
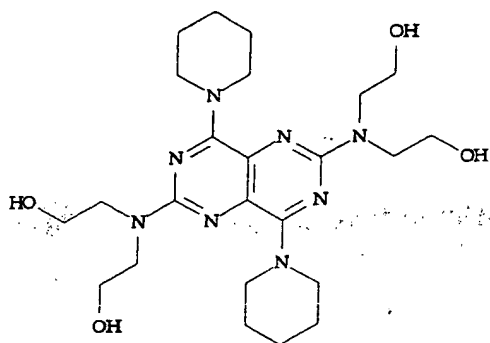
*D-107

Adenosine Uptake

DMI *(Desipramine, Desmethylimipramine hydrochloride)*

· HCl
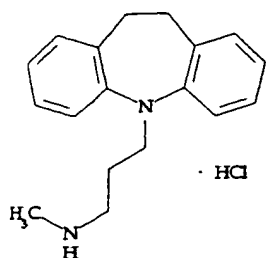
D-125

Norepinephrine Uptake

DNQX *(6,7-Dinitroquinoxaline-2,3-dione)*

*D-123

Kainic Acid

19

Domoic acid

COOH

CH₃

H

H₃C—COOH

COOH

N
H

*D-135
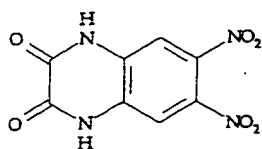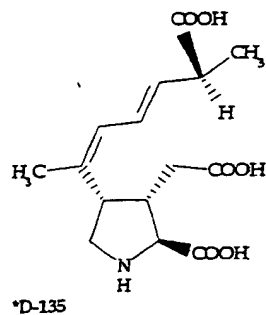
Kainic Acid

Dopamine *(3-Hydroxytyramine hydrochloride)*

· HCl

HO

HO

NH₂

D-019
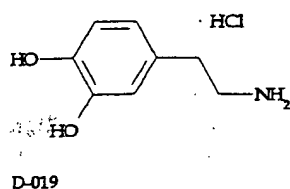
Clozapine
Dopamine *(Non-Selective)*
Dopamine₁
Dopamine₃ *(Rat Recombinant)*

DPDPE *([D-Pen^{2,5}]-Enkephalin)*

H-Tyr-D-Pen-Gly-Phe-D-Pen-OH

*E-119

Opiate, Non-Selective
Opiate, Mu *(Non-selective)*

DSLET *([D-Ser^{2}]-Leu-Enkephalin-Thr)*

Tyr-D-Ser-Gly-Phe-Leu-Thr

Opiate, Mu *(Non-selective)*
Opiate, Delta

DTG *(1,3-Di(2-tolyl)guanidine)*

H
N

H
N

CH₃

H₃C

N
H

*D-113
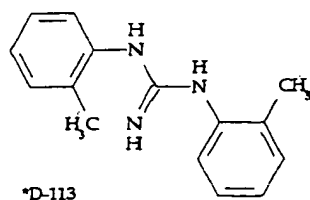
Sigma *(Non-Selective)*
Sigma₂

20

DuP753
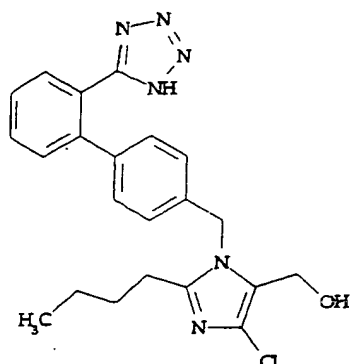
Angiotensin II, Type I *(Peripheral)*
Angiotensin II, Type 2 *(Central)*

Edrophonium chloride
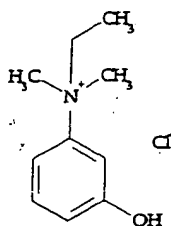
*E-102

Acetylcholinesterase

Efaroxan hydrochloride
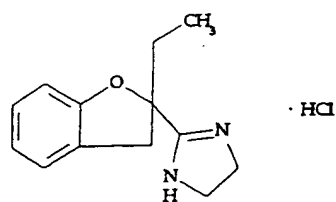
*E-110

Adrenergic, Alpha$_2$ *(Non-Selective)*
Adrenergic, Alpha$_{2A}$
Adrenergic, Alpha$_{2B}$

EGF *(Epidermal growth factor, murine)*

Asn-Ser-Tyr-Pro-Gly-Cys-Pro-Ser-Ser-Tyr-Asp-Gly-
Tyr-Cys-Leu-Asn-Gly-Gly-Val-Cys-Met-His-Ile-Glu-Ser-
Leu-Asp-Ser-Try-Thr-Cys-Asn-Cys-Val-Ile-Gly-Try-Ser-
Gly-Asp-Arg-Cys-Gln-Thr-Arg-Asn-Leu-Arg-Trp-Trp-
Glu-Leu-Arg

EGF *(Epidermal growth factor)*

EKC *(Ethylketocyclazocine)*
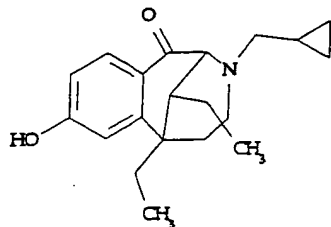
Opiate, Kappa

Eledoisin

pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met

Neurokinin, NK_1 *(Substance P)*
Neurokinin, NK_2 *(Neurokinin A)*
Neurokinin, NK_3 *(Neurokinin B)*

Endothelin-1 *(human, porcine)*

Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-
Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp

*E-134

Endothelin$_A$
Endothelin$_B$

Endothelin-2 *(human)*

Cys-Ser-Cys-Ser-Ser-Trp-Leu-Asp-Lys-Glu-Cys-
Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp

*E-135

Endothelin$_A$
Endothelin$_B$

Endothelin-3 *(rat)*

Cys-Thr-Cys-Phe-Thr-Tyr-Lys-Asp-Lys-Glu-
Cys-Val-Tyr-Tyr-Cys-His-Leu-Asp-Ile-Ile-Trp

*E-136

Endothelin$_A$
Endothelin$_B$

(±) Epibatidine dihydrochloride
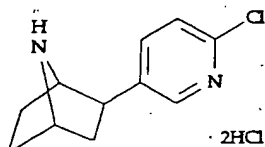
*E-127

Nicotinic

(+) Epibatidine-L-tartrate

H N

Cl

N

· C₄H₆O₆ → $\cdot\,C_4H_6O_6$

H
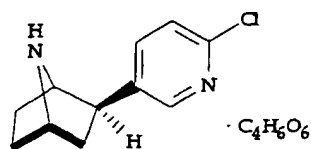
*E-129

Nicotinic

(-) Epibatidine-L-tartrate

H

Cl

N

HN

$\cdot\,C_4H_6O_6$

*E-130

Nicotinic

17β-Estradiol

HO

H₃C

HO
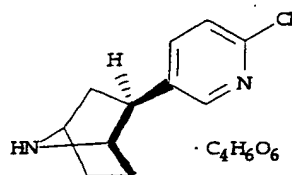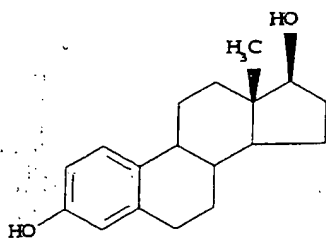
*E-125

Estradiol
Progesterone
Testosterone

Estriol

OH

H₃C

OH

HO
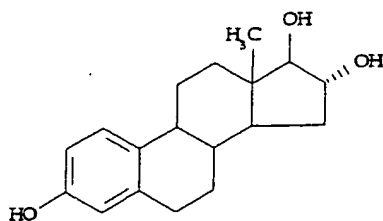
Progesterone
Estradiol

Ethyl-β-carboline-3-carboxylate (β-CCE)

O

O

CH₃

N

N
H
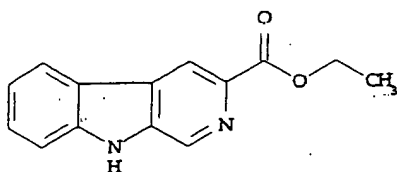
*E-001

Benzodiazepine (Central)

Fentanyl citrate

H$_3$C

·C$_6$H$_8$O$_7$
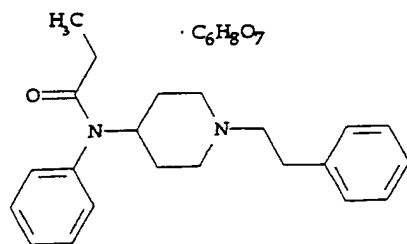
*F-108

Opiate, Mu

Fluoxetine

F$_3$C
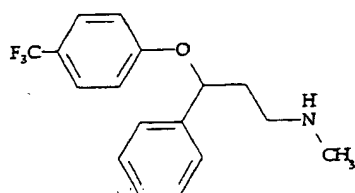
Serotonin Uptake

Fluphenazine dihydrochloride

OH

CF$_3$

2HCl
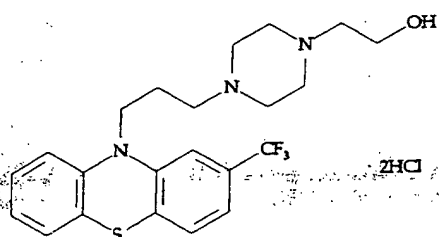
*F-101

Clozapine

Forskolin

CH$_3$

HO   CH$_3$ CH$_3$

CH$_2$

O

OH

O

H$_3$C   CH$_3$ OH   CH$_3$
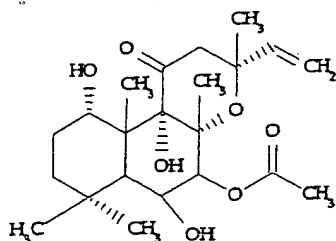
*F-105

Adenylate Cyclase (Forskolin)

Galanin (porcine)

Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-
Leu-Gly-Pro-His-Ala-Ile-Asp-Asn-His-Arg-
Ser-Phe-His-Asp-Lys-Tyr-Gly-Leu-Ala

Galanin

Galanin$_{(1-16)}$ agonist (porcine, rat)

Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-
Tyr-Leu-Leu-Gly-Pro-His-Ala-Ile

Galanin

24

Galantide

Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-
Leu-Gly-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met

Galanin

Gamma-aminobutyric acid

H_2N COOH

*G-012

Gamma-Aminobutyric Acid, GABA$_A$
Gamma-Aminobutyric Acid, GABA$_B$
Gamma-Aminobutyric Acid, GABA Uptake
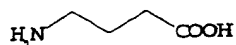
Gastrin I *(rat)*

pGlu-Arg-Pro-Pro-Met-Glu-Glu-Glu-
Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

Cholecystokinin *(Central, CCK$_B$)*

Glibenclamide *(Glyburide)*

*G-106

Potassium Channel, ATP-Modulated
(Glibenclamide)
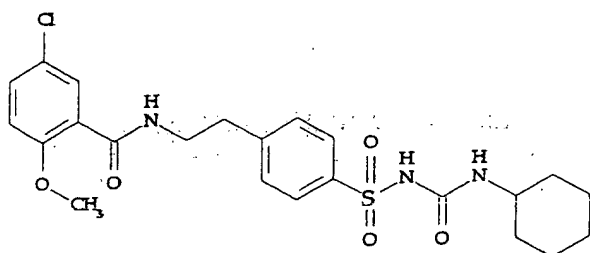
L-Glutamate hydrochloride

NH$_2$
HOO C COOH
H
· HCl
*G-100

AMPA
Glutamate *(Non-Selective)*
Glutamate Uptake
Kainic Acid
(+) MK-801
N-Methyl-D-Aspartate *(NMDA)*
Glutamic Acid Decarboxylase

Glycine

H_2N COOH
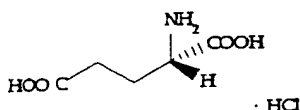
Glycine, Strychnine-Insensitive

---
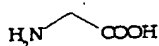
hGRP *(Gastrin releasing peptide, human)*

Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-
Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-
His-Trp-Ala-Val-Gly-His-Leu-Met

Gastrin Releasing Peptide *(GRP)*

---

h(Ac-Tyr^1,D-Phe^2]-GRF$_{(1-29)}$,VIP Antagonist

Acetyl-Tyr-D-Phe-Asp-Ala-Ile-Phe-Thr-Asn-
Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-
Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg

Vasoactive Intestinal Peptide *(VIP)*

25

GR113808

Serotonin$_4$

Guanabenz acetate *(WY-8678)*
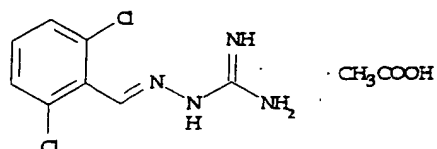
*G-110

Adrenergic, Alpha$_2$ *(Non-Selective)*
Adrenergic, Alpha$_{2A}$
Adrenergic, Alpha$_{2B}$
Imidazoline$_2$

Haloperidol
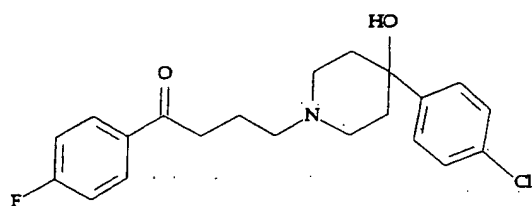
*H-100

Dopamine *(Non-Selective)*
Dopamine$_2$
Dopamine$_2$ *(Human Recombinant)*
Dopamine$_3$ *(Rat Recombinant)*
Sigma *(Non-Selective)*
Sigma$_1$
Sigma$_2$

Hemicholinium-3 dibromide
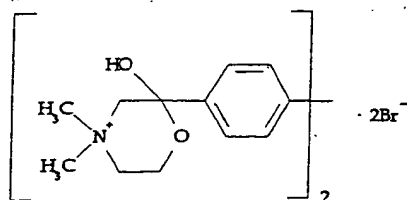
*H-108

Choline Uptake

HHSID *(Hexahydro-sila-difenidol hydrochloride)*
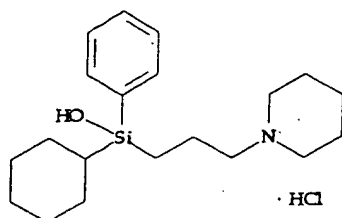
*H-126

Muscarinic$_2$
Muscarinic$_3$
Muscarinic$_2$ *(Human Recombinant)*
Muscarinic$_3$ *(Human Recombinant)*

p-F-HHSiD (p-F-Hexahydro-sila-difenidol hydrochloride)
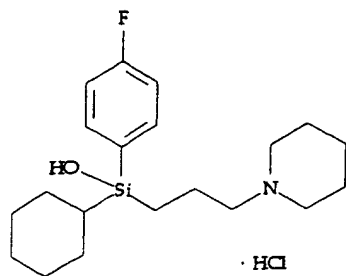
*H-127

Muscarinic$_1$ *(Human Recombinant)*
Muscarinic$_2$ *(Human Recombinant)*
Muscarinic$_3$ *(Human Recombinant)*
Muscarinic$_4$ *(Human Recombinant)*

Histamine dihydrochloride
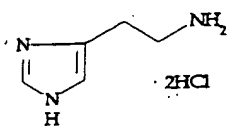
H-119

Histamine$_2$

HIV gp120 *(Fragment 421-438)*

Lys-Gln-Phe-Ile-Asn-Met-Trp-Gln-Glu-
Val-Gly-Lys-Ala-Met-Tyr-Ala-Pro-Pro

HIV gp120/CD$_4$

ICI-118,551 hydrochloride
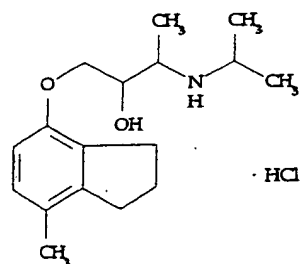
*I-127

Adrenergic, Beta$_1$
Adrenergic, Beta$_2$

ICI-89,406

Adrenergic, Beta$_1$
Adrenergic, Beta$_2$

27
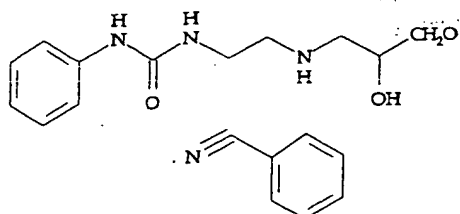
ICS-205-930 *(3-Tropanyl-indole-3-carboxylate methiodide)*

*T-113
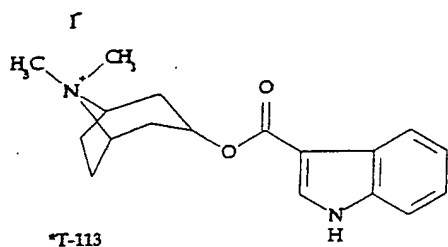
Serotonin$_3$

ICYP *(Iodocyanopindolol)*
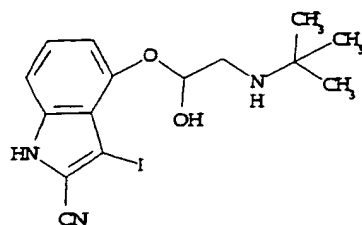
Serotonin$_{1B}$

Idazoxane hydrochloride *(RX 781094)*

*I-115
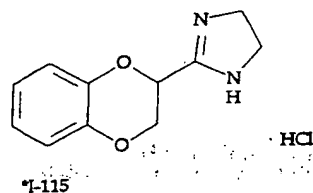
Imidazoline$_2$

IMETIT *(S-[2-(Imidazol-4-yl)ethyl)]isothiourea dihydrobromide)*

*I-135
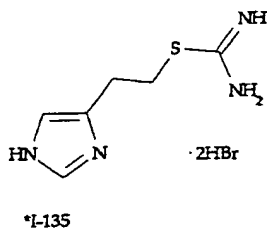
Histamine$_3$

Imipramine hydrochloride

*I-111
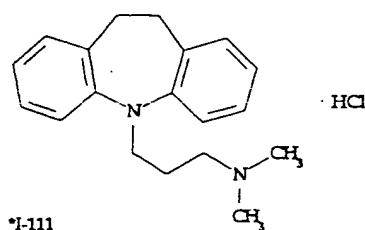
Serotonin Uptake
Norepinephrine Uptake
Monoamine Oxidase *(MAO$_B$)*

Inositol Phosphate, IP$_1$

Inositol Triphosphate, IP$_3$
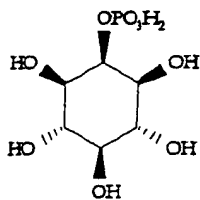
Inositol Phosphate, IP$_2$

Inositol Triphosphate, IP$_3$
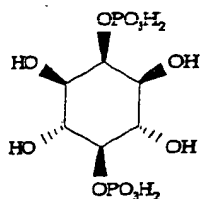
Inositol Phosphate, IP$_3$ hexasodium salt

*I-105

Inositol Triphosphate, IP$_3$
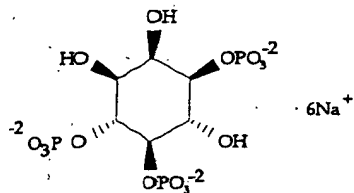
Inositol Phosphate, IP$_4$ tetrasodium salt
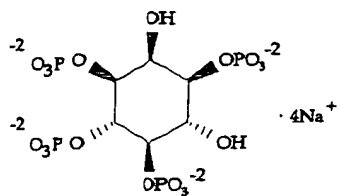
*I-113
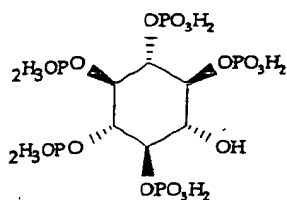
Inositol Triphosphate, IP$_3$

Inositol Phosphate, IP$_5$

Inositol Triphosphate, IP$_3$

Insulin *(porcine)*

Insulin

29

Interleukin-1α, human

Interleukin-1-α *(Human Recombinant)*

Isoguvacine hydrochloride

COOH

· HCl

N
H

*G-002
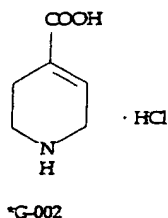
Gamma-Aminobutyric Acid, GABA$_A$

R(-)-Isoproterenol (+)-bitartrate salt

HO

OH

HO

CH$_3$

N

H

H

· C$_4$H$_6$O$_6$

CH$_3$

*I-102
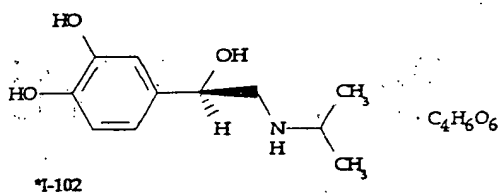
Adrenergic, Beta *(Non-Selective)*
Adrenergic, Beta$_1$
Adrenergic, Beta$_2$

Kainic acid

CH$_2$

H$_3$C

COOCH$_3$

N
H

COOCH$_3$
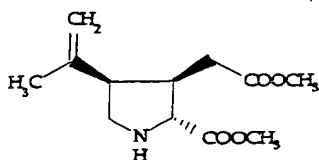
AMPA
Glutamate Uptake
Glutamate *(Non-Selective)*

Kainic acid dimethylester

CH$_2$

H$_3$C

COO

CH$_3$

N
H

COO

CH$_3$
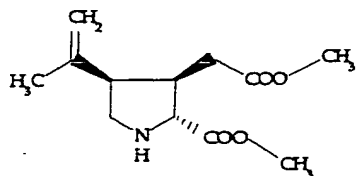
Kainic Acid

Kassinin

Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met

Neurokinin, NK$_1$ *(Substance P)*
Neurokinin, NK$_2$ *(Neurokinin A)*
Neurokinin, NK$_3$ *(Neurokinin B)*

Ketamine hydrochloride
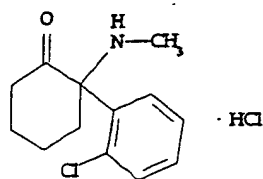
*K-101

Phencyclidine (PCP)

Ketanserin tartrate
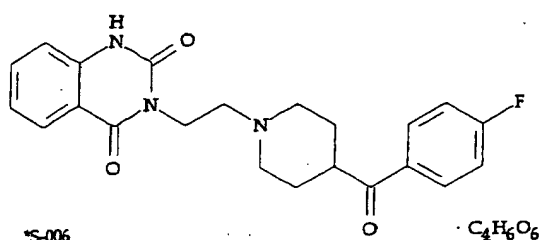
*S-006 $C_4H_6O_6$

Serotonin$_2$
Serotonin$_{2C}$

Kojic amine hydrobromide
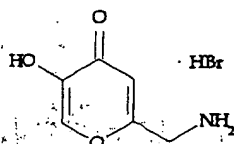
*K-104

Gamma-Aminobutyric Acid, GABA$_B$

L 364,718
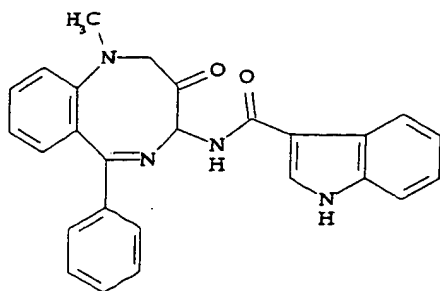
Cholecystokinin (Peripheral, CCK$_A$)
Cholecystokinin (Central, CCK$_B$)

L 365,260
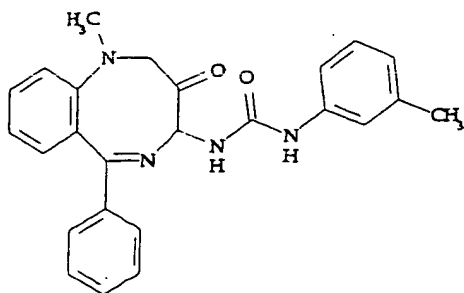
Cholecystokinin (Peripheral, CCK$_A$)
Cholecystokinin (Central, CCK$_B$)

Lidocaine hydrochloride
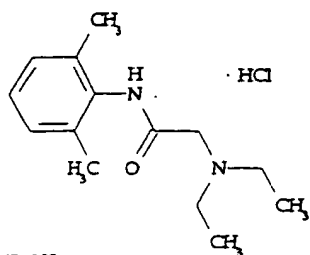
*L-102

Sodium Channel, Site 2 *(Batrachotoxin)*

Litorin

Glu-Gln-Trp-Ala-Val-Gly-His-Phe-Met

Gastrin Releasing Peptide *(GRP)*

Lorazepam
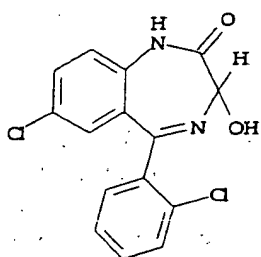
Benzodiazepine *(Central)*

Lorglumide
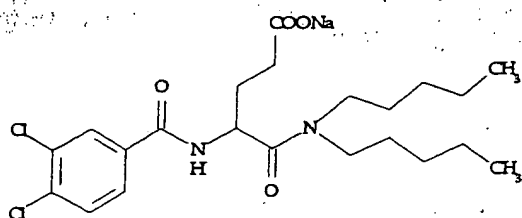
*L-109

Cholecystokinin *(Peripheral, CCK$_A$)*

D-LSD *(D-Lysergic acid diethylamide tartrate)*

·$C_4H_6O_6$
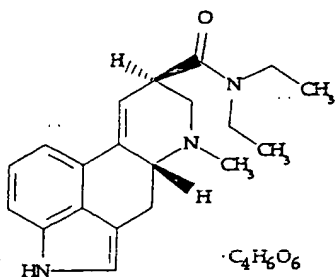
*L-114

Serotonin$_2$

LTB₄ *(Leukotriene B₄)*
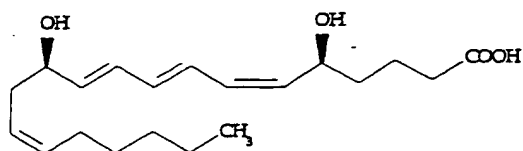
Leukotriene B₄ *(LTB₄)*

LTD₄ *(Leukotriene D₄)*
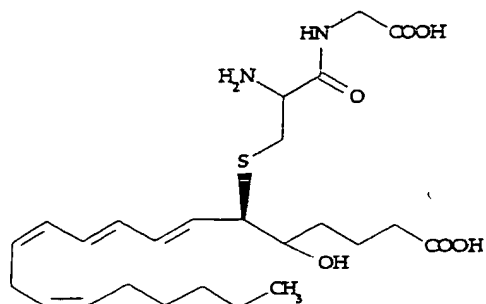
Leukotriene D₄ *(LTD₄)*

Mazindol
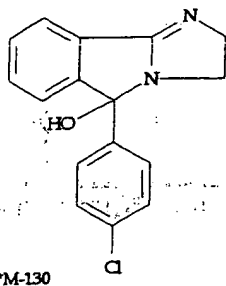
*M-130

Dopamine Uptake

MDL-72222 *(3-Tropanyl-3,5-dichlorobenzoate)*
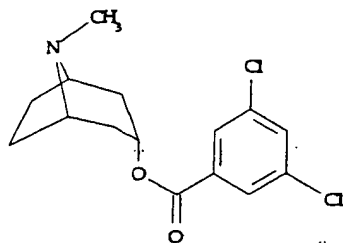
*T-102

Serotonin₃

Mecamylamine hydrochloride

*M-106

Nicotinic

33
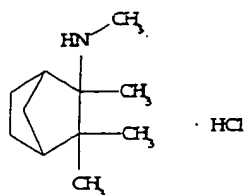
MECA *(5'-N-Methylcarboxamidoadenosine)*
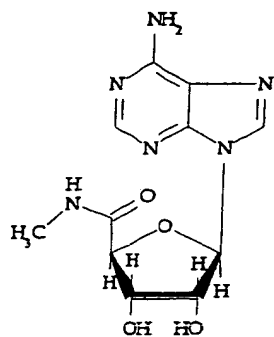
*A-024

Adenosine *(Non-Selective)*
Adenosine$_1$
Adenosine$_2$

Melatonin
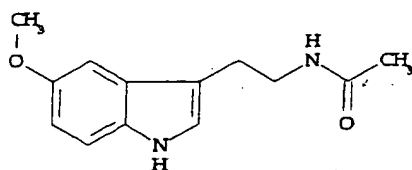
*M-113

Melatonin

Mesulgerine
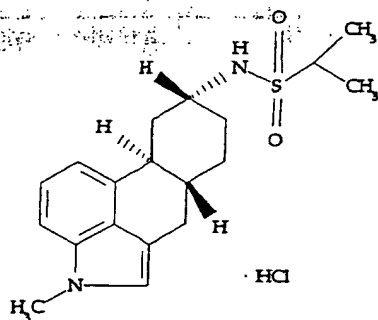
*M-153

Serotonin$_{1D}$
Serotonin$_{2C}$ *(formerly 1C)*
Serotonin$_7$ *(Rat Recombinant)*

Methiothepin *(Metitepine mesylate)*
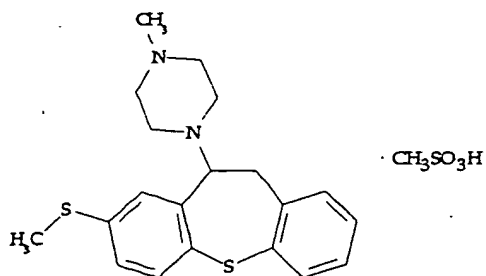
*M-149

Serotonin$_{1D}$
Serotonin$_4$
Serotonin$_6$ *(Rat Recombinant)*

34

Methoctramine tetrahydrochloride
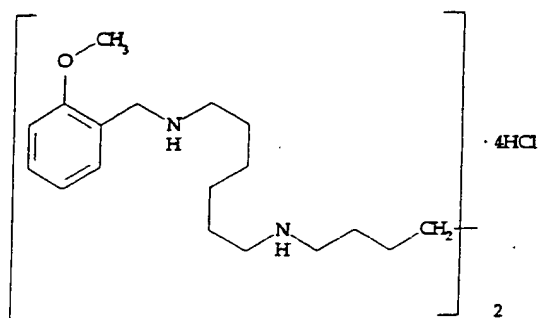
*M-105

Muscarinic$_1$
Muscarinic$_2$
Muscarinic$_3$
Muscarinic, Non-Selective *(Central)*
Muscarinic, Non-Selective *(Peripheral)*
Muscarinic, M$_1$ *(Human Recombinant)*
Muscarinic, M$_2$ *(Human Recombinant)*
Muscarinic, M$_3$ *(Human Recombinant)*
Muscarinic, M$_4$ *(Human Recombinant)*
Muscarinic, M$_5$ *(Human Recombinant)*
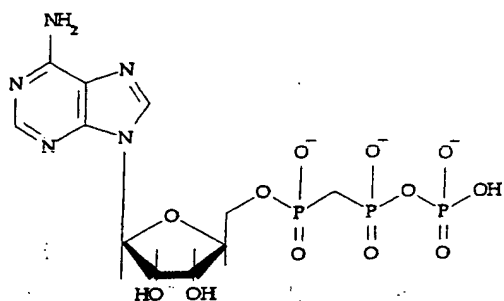
α,β-Methylene ATP

Purinergic, P$_{2Y}$

β,γ-Methylene ATP
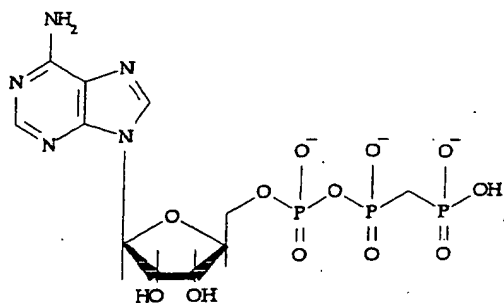
Purinergic, P$_{2Y}$

R(-)-α-Methylhistamine dihydrochloride
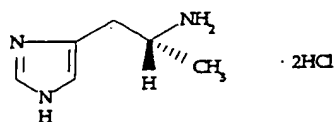
*H-128

Histamine$_3$

(-)-Methylscopolamine bromide
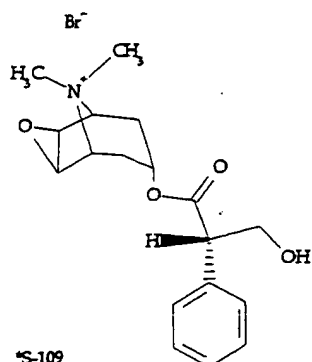
*S-109

Muscarinic$_1$ *(Human Recombinant)*
Muscarinic$_2$ *(Human Recombinant)*
Muscarinic$_3$ *(Human Recombinant)*
Muscarinic$_4$ *(Human Recombinant)*
Muscarinic$_5$ *(Human Recombinant)*

Methysergide maleate
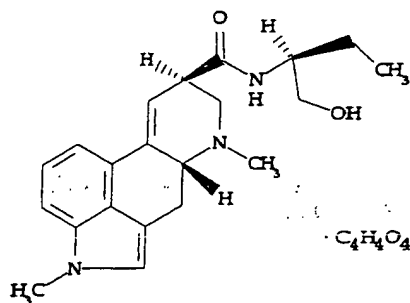
*M-137

Serotonin *(Non-Selective)*
Serotonin$_1$
Serotonin$_{1D}$
Serotonin$_2$
Serotonin$_{2C}$

Metoclopramide hydrochloride
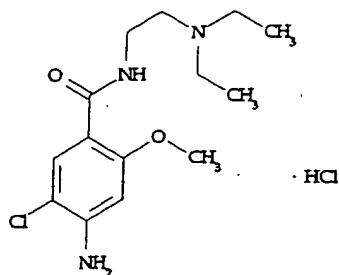
*M-117

Dopamine$_1$
Dopamine$_2$
Serotonin$_3$

Metoprolol
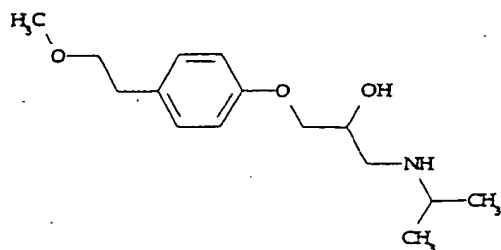
Adrenergic, Beta *(Non-Selective)*

Mianserin hydrochloride

H₃C

· HCl

*S-100
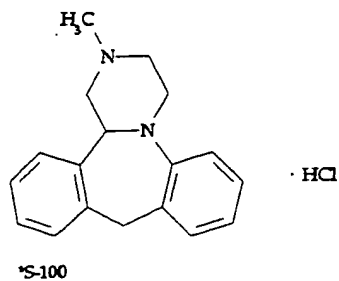
Serotonin *(Non-Selective)*
Serotonin$_1$
Serotonin$_{2C}$

Mibolerone

OH
H₃C
,,,,,,CH₃
CH₃

O
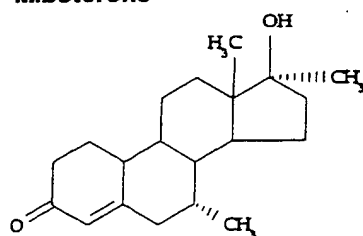
Testosterone

(+) MK-801 maleate

NH

· C$_4$H$_4$O$_4$

CH₃

*M-107
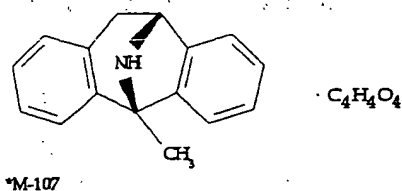
(+) MK-801
Phencyclidine *(PCP)*

Muscimol hydrobromide

HO

NH₂

N
O
· HBr

*G-019
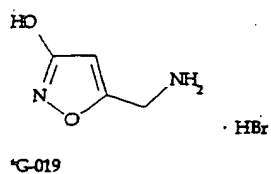
Gamma-Aminobutyric Acid, GABA$_A$
Gamma-Aminobutyric Acid, GABA$_B$

Nalbuphine hydrochloride

N
OH
· HCl

O
O
OH

*O-005
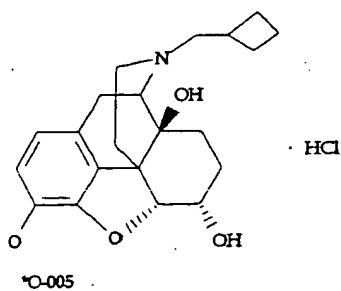
Opiate, Delta

Naloxone hydrochloride
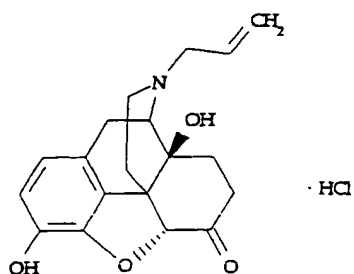
*O-002

Opiate *(Non-Selective)*
Opiate, Delta
Opiate, Mu *(Non-Selective)*

NBTI *(Nitrobenzylthioinosine)*
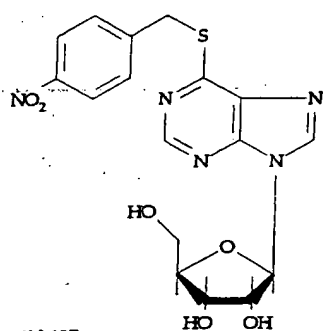
*N-127

Adenosine Uptake

NECA *(5'-N-Ethylcarboxamidoadenosine)*

*A-014

Adenosine *(Non-Selective)*
Adenosine$_1$
Adenosine$_2$

Neomycin
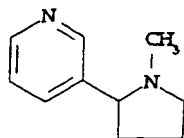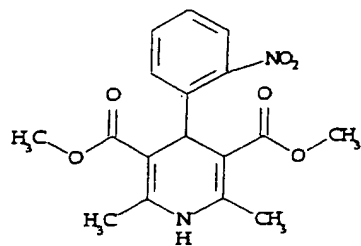
Neomycin B
$R_1=H$
$R_2=CH_2NH_2$

Neomycin C
$R_1=CH_2NH_2$
$R_2=H$

Calcium Channel, Type N *(ω-Conotoxin)*

α-NETA (2-[α-Naphthoyl]ethyltrimethylammonium iodide)

Choline Acetyltransferase

Neurotensin

Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg- Pro-Tyr-Ile-Leu

Neurotensin

Neurotensin (8-13)

Arg-Arg- Pro-Tyr-Ile-Leu

Neurotensin

Neurotensin (10-13)

Pro-Tyr-Ile-Leu

Neurotensin

Nicotine

Nicotinic

*N-114

Nifedipine

Calcium Channel, Type T&L *(Nitrendipine)*

*N-114

(±)-Niguldipine hydrochloride
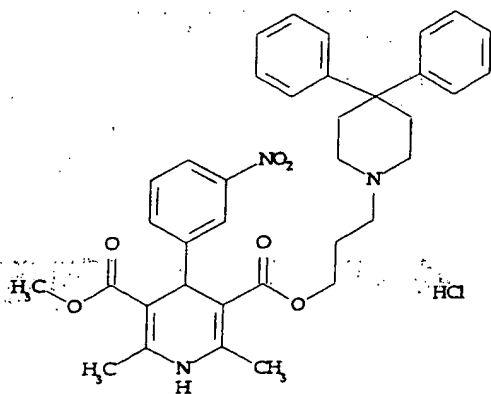
Adrenergic, Alpha$_{1B}$
Calcium Channel, Type T&L *(Nitrendipine)*

*N-134

(±)-Nipecotic acid
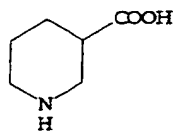
Gamma-Aminobutyric Acid Uptake, GABA Uptake
Gamma-Aminobutyric Acid, GABA$_A$

*G-005

Nisoxetine hydrochloride *(LY-94,939)*
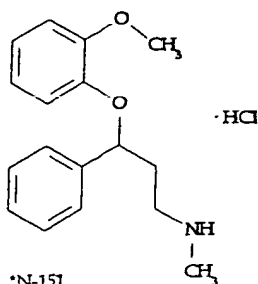
Norepinephrine Uptake

*N-151

NK$_A$ *(Neurokinin$_A$)*

His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met

*N-146

Neurokinin, NK$_2$ *(Neurokinin$_A$)*
Neurokinin, NK$_3$ *(Neurokinin B)*

NK$_B$ *(Neurokinin$_B$)*

Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met

*N-148

Neurokinin, NK$_3$ *(Neurokinin B)*

NMDA   *(N-Methyl-D-aspartic acid)*
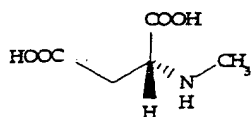
*M-102

Glutamate *(Non-Selective)*
(+) MK-801
N-Methyl-D-Aspartate *(NMDA)*

NOARG *(N^G-Nitro-L-arginine)*
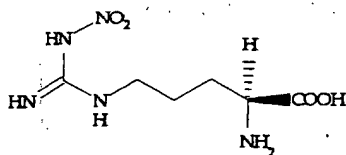
*A-160

Nitric Oxide Synthase, Constitutive, Neuronal *(NOS)*

Nomifensine maleate

· C$_4$H$_4$O$_4$
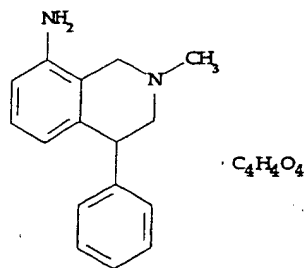
*N-123

Dopamine Uptake

(±)-Norepinephrine hydrochloride

· HCl
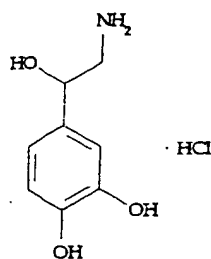
*N-112

Adrenergic, Alpha$_2$ *(Non-Selective)*

NPPB *(5-Nitro-2-[3-phenylpropylamino] benzoic acid)*

*N-150

Chloride Channel, TBOB
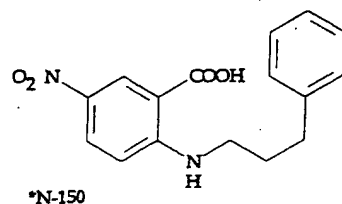
NPY *(Neuropeptide Y, porcine)*

Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-
Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-
Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-
Ile-Thr-Arg-Gln-Arg-Tyr

Neuropeptide Y *(Non-Selective)*

Oxymetazoline hydrochloride
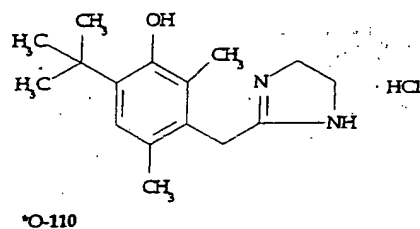
*O-110

Adrenergic, Alpha$_{2A}$
Adrenergic, Alpha$_{2B}$
Adrenergic, Alpha$_{2A}$ (Human recombinant)
Adrenergic, Alpha$_{2B}$ (Human recombinant)

Oxytocin

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly

*O-117

Oxytocin
Vasopressin$_1$

[Thr^4,Gly^7] -Oxytocin

Cys-Tyr-Ile-Thr-Asn-Cys-Gly-Leu-Gly

Oxytocin

PACPX
(1,3-Dipropyl-8-[2-amino-4-chlorophenyl]-xanthine)

*A-015

Adenosine$_1$
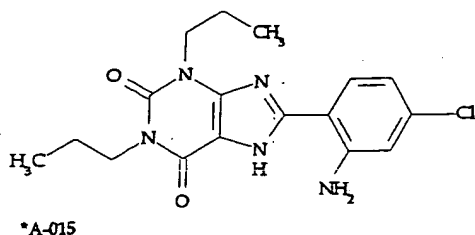
C₁₆-PAF *(C₁₆-Platelet activating factor)*
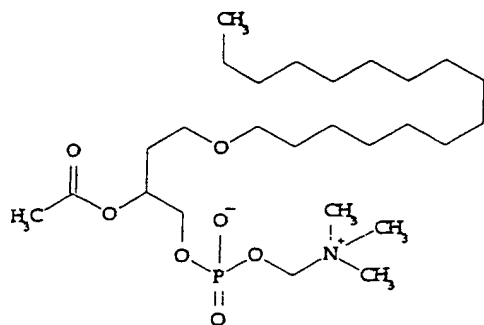
Platelet Activating Factor *(PAF)*

Pargyline
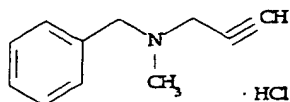
Monoamine Oxidase *(MAO_B)*

*D-026

Paroxetine
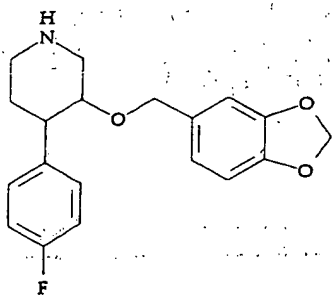
Serotonin Uptake

PCP *(Phencyclidine hydrochloride)*
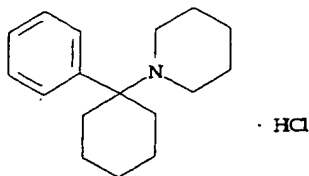
(+) MK-801
Phencyclidine *(PCP)*

*P-123

PDA *(Phorbol 12, 13-diacetate)*
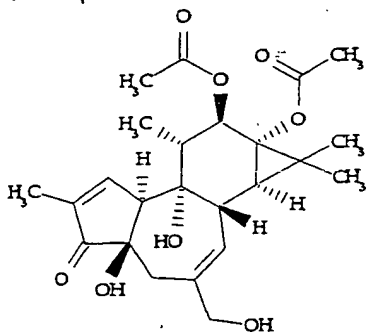
Protein Kinase C *(PDBU)*

*P-146

PDBu *(Phorbol 12, 13-dibutyrate)*
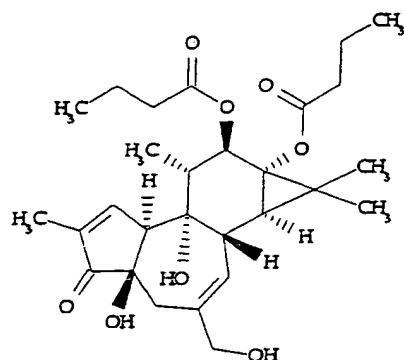
Protein Kinase C *(PDBU)*

*P-147

L-*trans*-2,4-PDC
(L-trans-Pyrollidine-2,4-dicarboxylic acid)
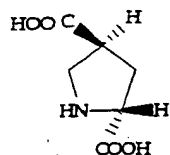
Glutamate Uptake

*P-167

P-PDGF AB *(P-Platelet derived growth factor (AB))*

n/a

Platelet Derived Growth Factor *(PDGF)*

(+)-Pentazocine hydrochloride
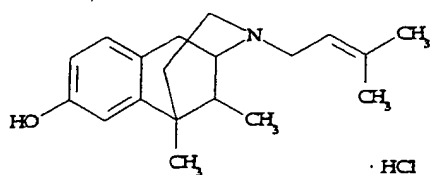
Opiate, Delta
Sigma *(Non-Selective)*

*P-144

Phentolamine mesylate
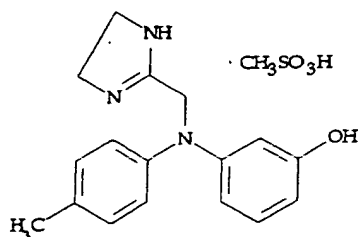
Adrenergic, Alpha$_1$ *(Non-Selective)*
Adrenergic, Alpha$_2$ *(Non-Selective)*

*P-131

Phenylpiperazine
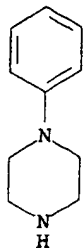
Serotonin$_{1B}$

Physalaemin

Glu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met

Neurokinin, NK$_1$ *(Substance P)*
Neurokinin, NK$_2$ *(Neurokinin A)*
Neurokinin, NK$_3$ *(Neurokinin B)*

(-)-Physostigmine *(Eserine)*

*P-155
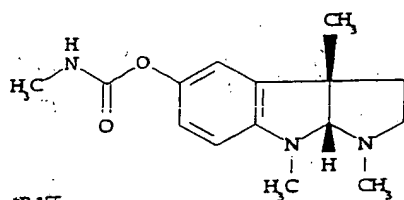
Acetylcholinesterase

Picrotoxin
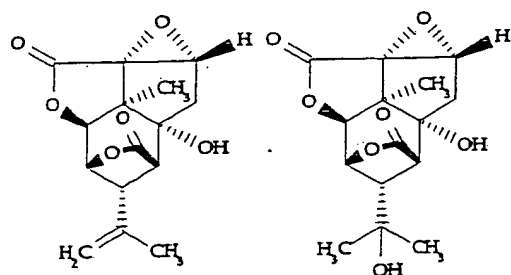
Picrotoxinin   Picrotin

*P-117

Chloride Channel *(TBOB)*

Pinane Thromboxane

Thromboxane A$_2$

45
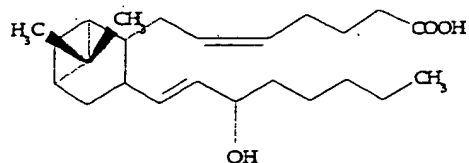
Pindolol
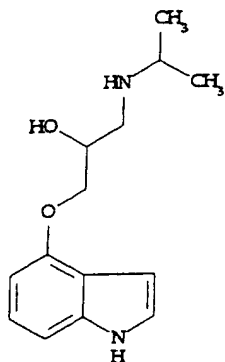
*P-125

Adrenergic, Beta *(Non-Selective)*

Pirenzepine hydrochloride

2HCl
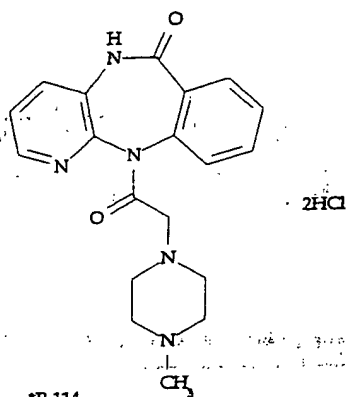
*P-114

Muscarinic, Non-selective *(Central)*
Muscarinic$_1$
Muscarinic$_1$ *(Human Recombinant)*
Muscarinic$_2$ *(Human Recombinant)*
Muscarinic$_3$ *(Human Recombinant)*
Muscarinic$_4$ *(Human Recombinant)*
Muscarinic$_5$ *(Human Recombinant)*

PK 11195
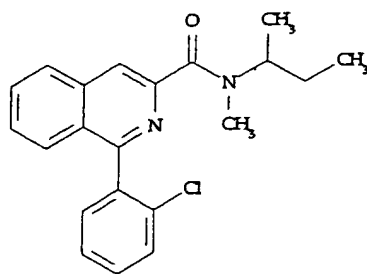
*P-130

Benzodiazepine *(Peripheral)*

PMA (4α-Phorbol 12-myristate 13-acetate)

Protein Kinase C *(PDBU)*

*P-148
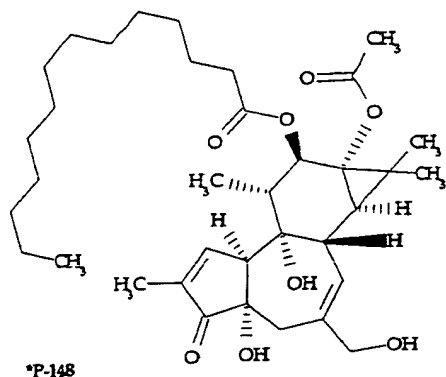
Prazosin hydrochloride

Adrenergic, Alpha$_1$ *(Non-Selective)*
Adrenergic, Alpha$_{1A}$
Adrenergic, Alpha$_{1B}$
Adrenergic, Alpha$_{2A}$
Adrenergic, Alpha$_{2B}$
Adrenergic, Alpha$_{2A}$ *(Human recombinant)*
Adrenergic, Alpha$_{2C}$ *(Human recombinant)*
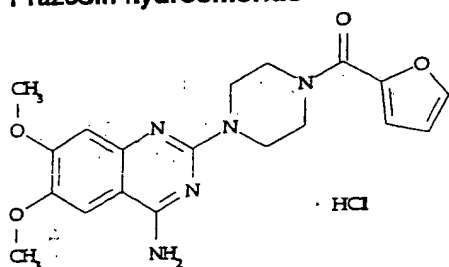
*P-115

Procainamide hydrochloride

Sodium Channel, Site 2 *(Batrachotoxin)*
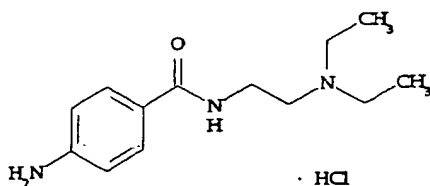
*P-137

Procaine hydrochloride

Sodium Channel, Site 2 *(Batrachotoxin)*

Progesterone

H₃C → H_3C
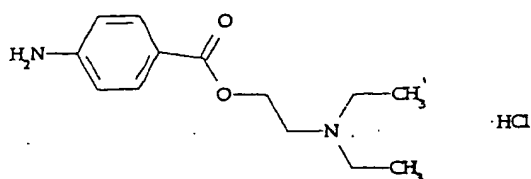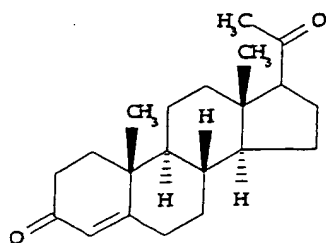
P-165

Estradiol
Testosterone
Progesterone

Promegestone
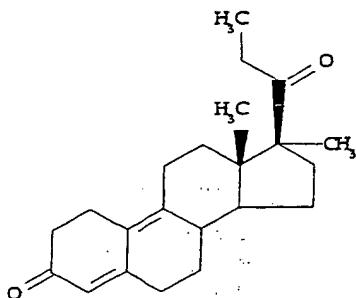
Progesterone

(±) Propranolol hydrochloride

· HCl
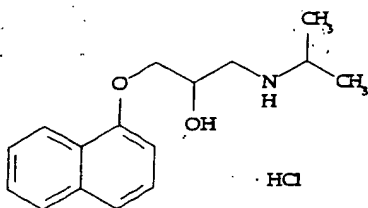
P-128

Adrenergic, Beta *(Non-Selective)*

Pyrilamine maleate

· $C_4H_4O_4$
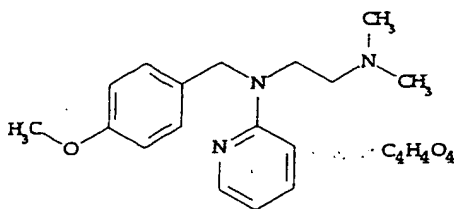
P-129

Histamine₁

Histamine$_1$

PYY *(Peptide YY, porcine, rat)*

Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-
Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Ser-
Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-
Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr

Neuropeptide Y *(NPY)*

(+)-QNB *(Quinuclidinyl benzilate)*

Muscarinic, Non-Selective *(Central)*
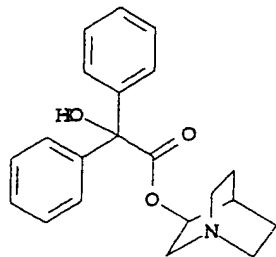
*C-002

Quinidine

Sodium Channel, Site 1 *(Saxitoxin)*
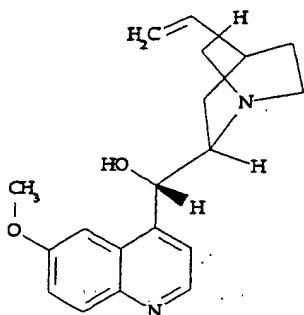
(-)-Quinpirole hydrochloride *(LY-171,555)*

· HCl

*Q-102

Dopamine$_2$
Dopamine$_3$
Dopamine$_3$ *(Rat Recombinant)*

Quipazine dimaleate

· 2C$_4$H$_4$O$_4$
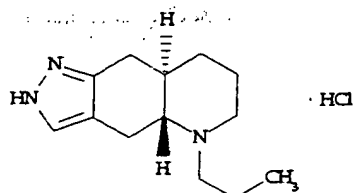
*S-007

Serotonin$_{1B}$
Serotonin$_{1D}$
Serotonin$_3$
Serotonin Uptake
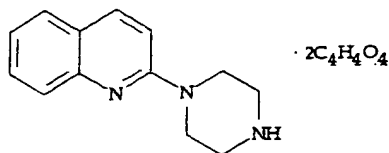
(+)- Quisqualic acid

*Q-103

AMPA
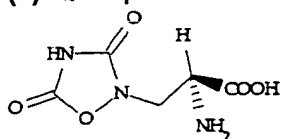
R1881 *(Methyltrienolone)*

Testosterone
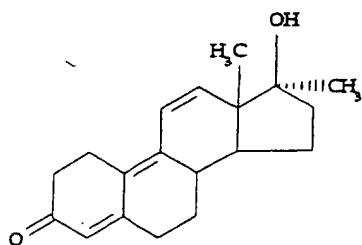
Ranatensin

Glu-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met

Gastrin Releasing Peptide *(GRP)*

Rauwolscine hydrochloride

*R-104

Adrenergic, Alpha$_{2A}$
Adrenergic, Alpha$_{2B}$
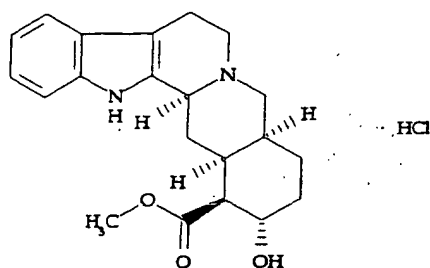
Renzapride

Serotonin$_4$

Risperidone
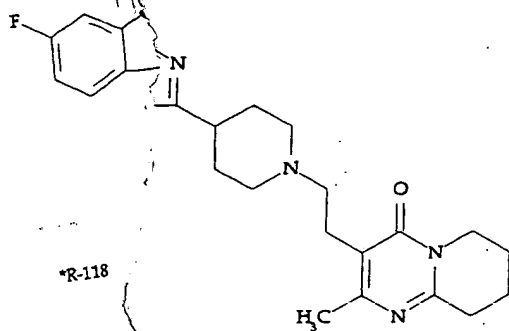
*R-118

Serotonin$_{1D}$

Ritanserin

Serotonin$_2$

H$_3$C
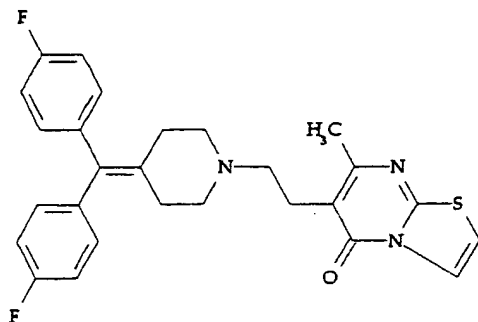
*R-103

Ro 05-4864 *(4'-Chlorodiazepam)*

Benzodiazepine *(Peripheral)*

H$_3$C
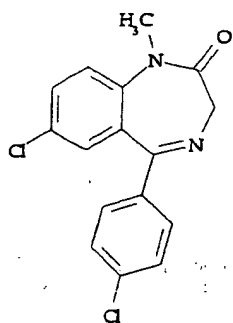
*C-140

Ro 15-1788 *(Flumazinil)*

Benzodiazepine *(Central)*
Benzodiazepine *(Peripheral)*

CH$_3$

CH$_3$
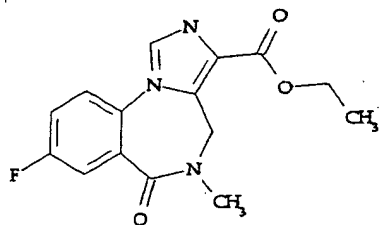
Ro 16-6491 hydrochloride

Monoamine Oxidase *(MAO$_B$)*

·HCl

NH$_2$
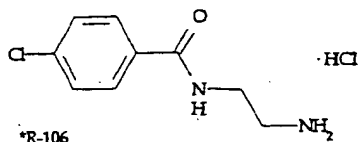
*R-106

Ro 41-1049 hydrochloride

Monoamine Oxidase *(MAO$_A$)*

NH$_2$

·HCl
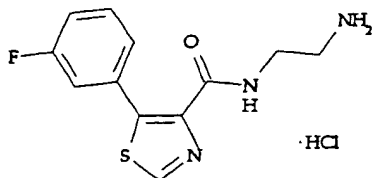
*R-107

RU 24969

Serotonin$_{1A}$
Serotonin$_{1B}$

RU 24989

Serotonin$_1$

RX 781094 hydrochloride

·HCl

*I-115

Adrenergic, Alpha$_2$ *(Non–Selective)*
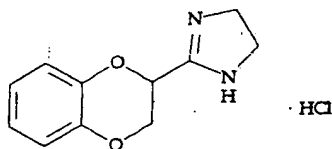
RX 821002 hydrochloride

· HCl

*R-105

Adrenergic, Alpha$_2$ *(Non–Selective)*
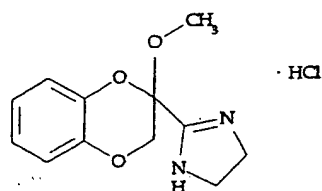
Saxitoxin

Calcium Channel, Type T&L *(Nitrendipine)*
Sodium Channel, Site 1 *(Saxitoxin)*
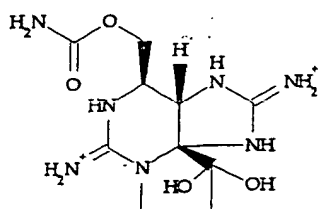
R(+)-SCH 23390 hydrochloride *(R(+)-CHMB)*
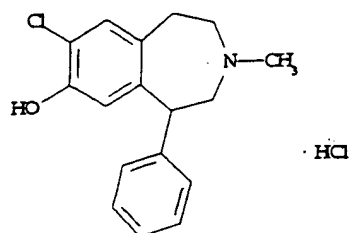
*D-054

Dopamine *(Non-Selective)*
Dopamine$_1$
Dopamine$_2$
Dopamine$_3$ *(Rat Recombinant)*

(-)-Scopolamine hydrobromide
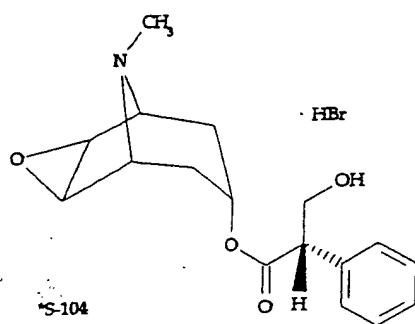
*S-104

Muscarinic, Non-Selective *(Central)*
Muscarinic, Non-Selective *(Peripheral)*

D-Serine
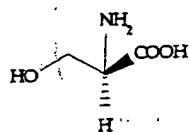
*S-135

Glycine, Strychnine-Insensitive

L-Serine
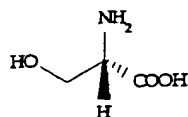
Glycine, Strychnine-Insensitive

Sertindole

Serotonin$_{1D}$

(+)-SKF-10047 *((+)-N-Allylnormetazocine hydrochloride)*
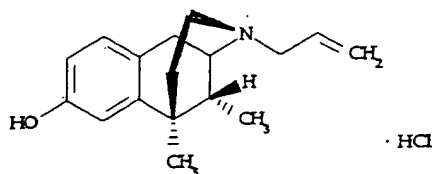
*A-114

Sigma *(Non-Selective)*

(+)-SKF 38393 hydrochloride
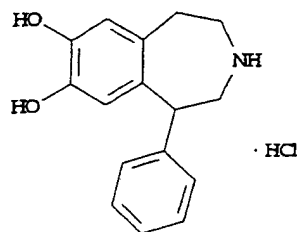
*D-047

Dopamine[1]
Dopamine[2]
Dopamine *(Non-Selective)*

Somatostatin

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-
Thr-Phe-Thr-Ser-Cys

Somatostatin

[Tyr⁰,D-Trp⁸]-Somatostatin

$[Tyr^0, D\text{-}Trp^8]$-Somatostatin

Tyr-Ala-Gly-Cys-Lys-Asn-Phe-Phe-D-Trp-
Lys-Thr-Phe-Thr-Ser-Cys

Somatostatin

Somatostatin 28

Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-
Arg-Glu-Arg-Lys-Ala-Gly-Cys-Lys-Asn-Phe-
Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

Somatostatin

Spiperone hydrochloride
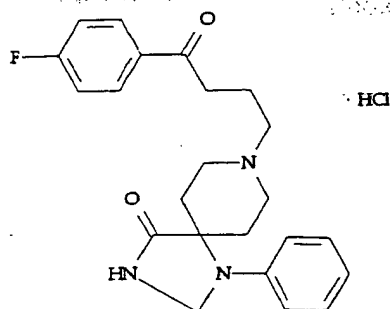
*D-050

Dopamine *(Non-Selective)*
Dopamine[1]
Dopamine[2]
Serotonin, 5HT *(Non-Selective)*

Spiroxatrine *(R 5188)*
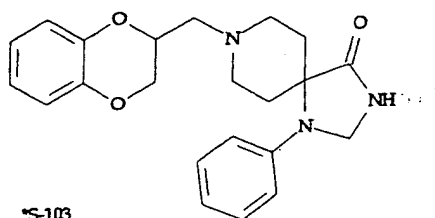
*S-103

Serotonin[1A]

SQ 29,548

*S-144
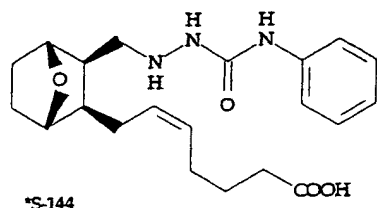
Thromboxane A$_2$

Strychnine hydrochloride

· HCl

*S-124
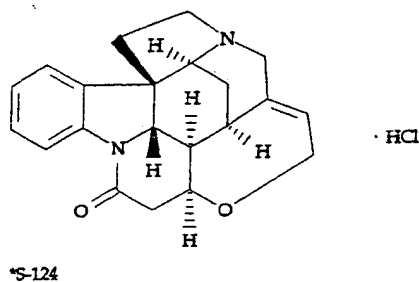
Glycine, Strychnine-Sensitive

Substance P

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met

Neurokinin, NK$_1$ *(Substance P)*
Neurokinin, NK$_2$ *(Neurokinin A)*
Neurokinin, NK$_3$ *(Neurokinin B)*

Substance P$_{4-11}$

Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met

Neurokinin, NK$_1$ *(Substance P)*

(±)-Sulpiride

*S-116
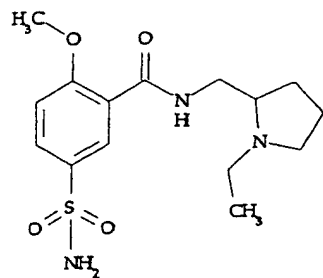
Dopamine$_2$

Sumatriptan
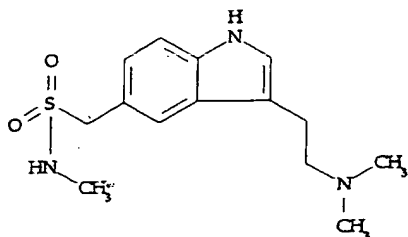
Serotonin$_{1D}$

TBPS *(tert-butyl-bicyclc[2,2,2]phosphorothionate)*
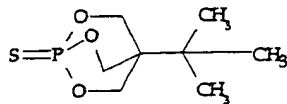
*B-104

Chloride Channel *(TBOB)*

TEA chloride *(Tetraethylammonium chloride)*
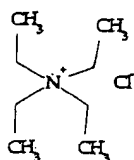
*T-105

Potassium Channel, Voltage Dependent
   (Charybdotoxin)
Potassium Channel, Low Conduct. Ca^{2+} Activated
   (Apamin)
Potassium Channel, ATP-Modulated *(Glibenclamide)*

Telenzepine dihydrochloride

·2HCl
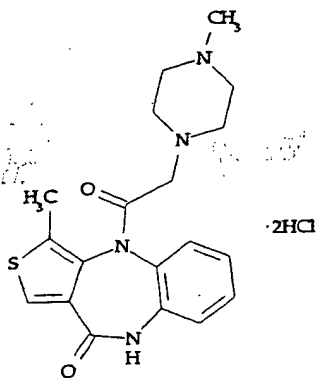
*T-122

Muscarinic$_1$
Muscarinic$_1$ *(Human Recombinant)*

Testosterone
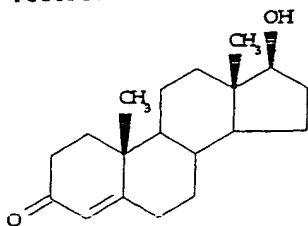
*T-130

Estradiol
Progesterone
Testosterone

Tetracaine hydrochloride
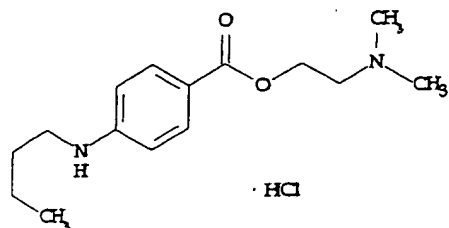
*T-114

Sodium Channel, Site 2 *(Batrachotoxin)*

Tetrodotoxin
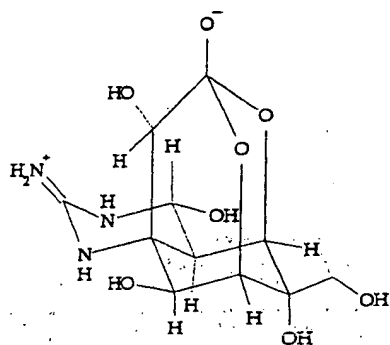
*T-127

Sodium Channel, Site 1 *(Saxitoxin)*

TFMPP *(N-(3-Trifluoromethylphenyl) piperazine hydrochloride)*
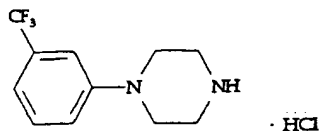
*S-005

Serotonin$_{1B}$

Thioperamide maleate *(MR 12842)*
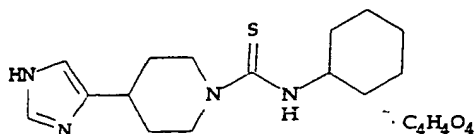
*T-123

Histamine$_3$

THIP *(4,5,6,7-Tetrahydroisoxazolo [5,4-c]pyridin-3-ol hydrochloride)*
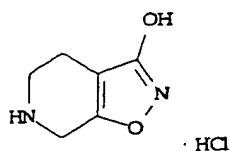
*T-101

Gamma-Aminobutyric Acid, GABA$_A$

Tiotidine
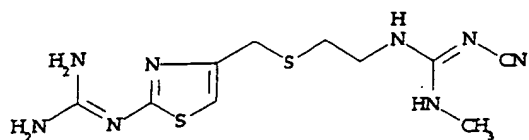
Histamine$_2$

rTNFα *(Recombinant tumor necrosis factor, human)*

Tumor Necrosis Factor *(TNF)*

TRH *(Thyrotropin releasing hormone)*

Glu-His-Pro

Thyrotropin Releasing Hormone *(TRH)*

*T-168

Triazolam

Benzodiazepine *(Central)*
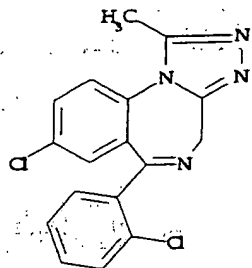
*T-129

Triprolidine hydrochloride

Histamine$_1$
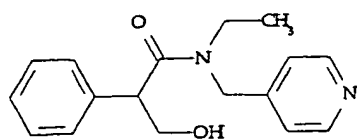
· HCl

*T-118

Tropicamide

Muscarinic$_1$ *(Human Recombinant)*
Muscarinic$_2$ *(Human Recombinant)*
Muscarinic$_3$ *(Human Recombinant)*
Muscarinic$_4$ *(Human Recombinant)*
Muscarinic$_5$ *(Human Recombinant)*

Tryptamine hydrochloride

*T-109

Serotonin$_{1D}$
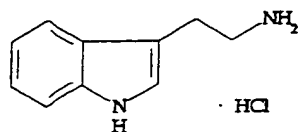
(+)-Tubocurarine dichloride

T-110

Nicotinic
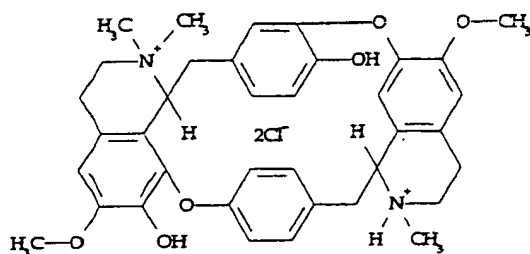
U-46619 (9,11-Dideoxy-11α, 9 α-epoxy-methanoprostaglandin)

Thromboxane
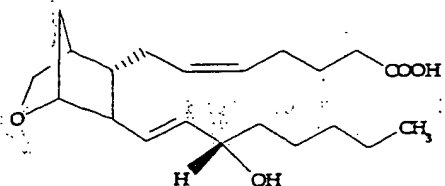
(±)-U-50488 methanesulfonate

*U-102

Opiate, Mu *(Non-Selective)*
Opiate, Kappa
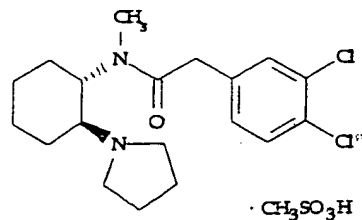
U-69593

*U-103

Opiate, Kappa
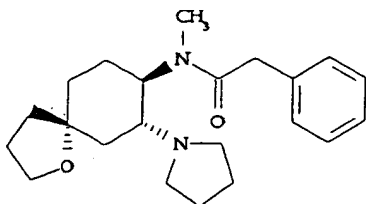
UK 14,304
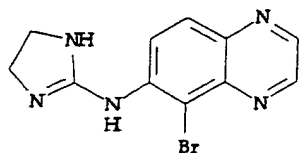
*U-104

Adrenergic, Alpha$_{2A}$
Adrenergic, Alpha$_{2B}$

Urapidil
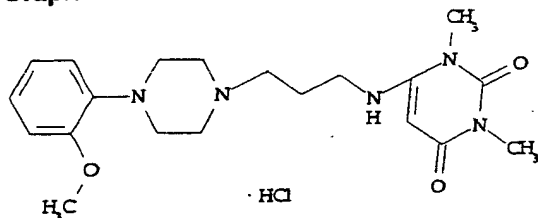
Adrenergic, Alpha$_1$

---

r/h/p VIP *(Vasoactive intestinal peptide,
rat/human/porcine)*

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-
Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-
Val-Lys-Lys-Try-Leu-Asn-Ser-Ile-Leu-Asn

Vasoactive Intestinal Peptide *(VIP)*

VIP,4CL, VIP Antagonist *([p-Chloro-D-Phe[6], Leu[17]]
Vasoactive intestinal peptide, antagonist)*

His-Ser-Asp-Ala-Val-4-Cl-D-Phe-Thr-Asp-
Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Leu-Ala-
Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn

Vasoactive Intestinal Peptide *(VIP)*

VIP $_{1-12}$ *(Vasoactive intestinal peptide,
fragment 1-12)*

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-
Tyr-Thr-Arg

Vasoactive Intestinal Peptide *(VIP)*

VIP $_{10-28}$ *(Vasoactive intestinal peptide,
fragment 10-28)*

Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-
Val-Lys-Lys-Try-Leu-Asn-Ser-Ile-Leu-Asn

Vasoactive Intestinal Peptide *(VIP)*

[Arg^8]-Vasopressin *(AVP)*

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly

Oxytocin
Vasopressin$_1$
Vasopressin$_2$

Desmopressin *(dDAVP)*
(Desamino^1, D-Arg^8]-Vasopressin)

Mpr-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly

Oxytocin
Vasopressin$_1$

[Lsy[8]]-Vasopressin (LVP)	Vasopressin$_1$
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly	

[Phe[2],Ile[3],Orn[8]]-Vasopressin	Vasopressin$_1$
Cys-Phe-Ile-Gln-Asn-Cys-Pro-Orn-Gly	

[d(CH$_2$)$_5$[1], D-Ile[2], Ile[4], Arg[8]]- Vasopressin	Vasopressin$_2$
Pmp-D-Ile-Phe-Ile-Asn-Cys-Pro-Arg-Gly	

[d(CH$_2$)$_5$[1], D-Ile[2], Ile[4], Arg[8], Ala[9]]- Vasopressin	Vasopressin$_2$
Pmp-D-Ile-Phe-Ile-Asn-Cys-Pro-Arg-Ala	

(±)-Verapamil hydrochloride

•V-102

Calcium Channel, Type T&L *(Nitrendipine)*
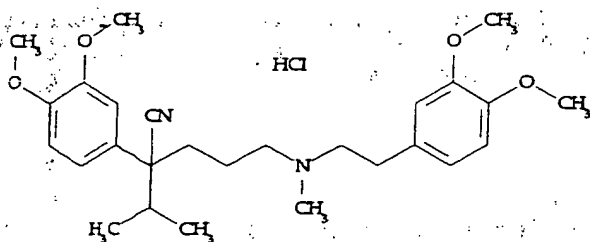
Veratridine

•V-109

Sodium Channel, Site 2 *(Batrachotoxin)*
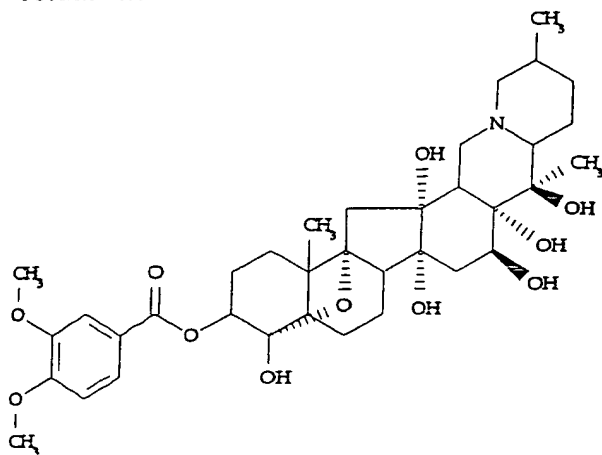
WB-4101 hydrochloride
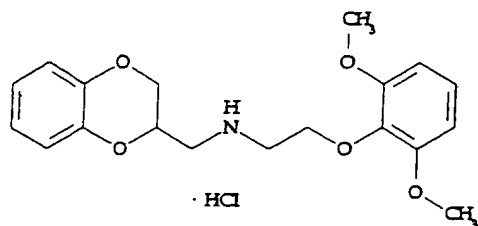
*B-018

Adrenergic, Alpha$_1$ *(Non-Selective)*
Adrenergic, Alpha$_{1A}$
Adrenergic, Alpha$_{1B}$

Yohimbine hydrochloride
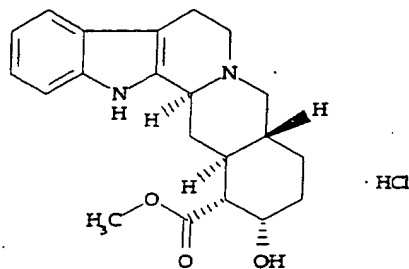
*Y-100

Adrenergic, Alpha$_1$ *(Non-Selective)*

Zimelidine dihydrochloride
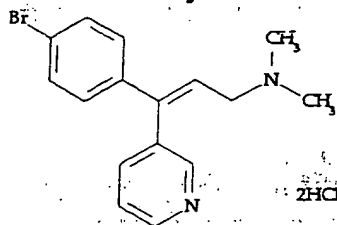
*Z-101

Serotonin Uptake

Catnum	Name	Position
A-001	7-(beta-Chloroethyl)theophylline	RK001-A2
A-003	1,3-Diethyl-8-phenylxanthine	RK001-B2
A-004	Theophylline	RK001-C2
A-005	1,7-Dimethylxanthine	RK001-D2
A-006	Theobromine	RK001-E2
A-007	3-Isobutyl-1-methylxanthine	RK001-F2
A-009	R(-)-N6-(2-Phenylisopropyl)adenosine	RK001-G2
A-010	Caffeine	RK001-H2
A-013	8-(p-Sulfophenyl)theophylline	RK001-A3
A-014	5'-N-Ethylcarboxamidoadenosine	RK001-B3
A-016	Adenosine	RK001-C3
A-017	N6-Cyclopentyladenosine	RK001-D3
A-018	3-n-Propylxanthine	RK001-E3
A-019	2-Chloroadenosine	RK001-F3
A-023	2-Methylthioadenosine triphosphate tetrasodium	RK001-G3
A-024	5'-N-Methyl carboxamidoadenosine	RK001-H3
A-025	1-Methylisoguanosine	RK001-A4
A-107	Aminophylline	RK001-B4
A-111	Adenosine amine congener	RK001-C4
A-145	1-Allyl-3,7-dimethyl-8-p-sulfophenylxanthine	RK001-D4
A-236	AB-MECA	RK001-E4
A-242	Alloxazine	RK001-F4
B-101	N6-Benzyladenosine	RK001-G4
B-152	N6-Benzyl-5'-N-ethylcarboxamidoadenosine	RK001-H4
C-101	8-Cyclopentyl-1,3-dipropylxanthine	RK001-A5
C-103	5'-(N-Cyclopropyl)carboxamidoadenosine	RK001-C5
C-141	CGS-21680 hydrochloride	RK001-D5
C-142	2-Chloro- N6-cyclopentyladenosine	RK001-E5
C-145	2-Chloro-adenosine triphosphate tetrasodium	RK001-F5
C-179	Chlorpropamide	RK001-G5
C-187	Chlorzoxazone	RK001-H5
C-192	Chlomezanone	RK001-A6
C-197	8-(3-Chlorostyryl)caffeine	RK001-B6
C-199	CGS-15943	RK001-C6
D-107	Dipyridamole	RK001-D6
D-117	Disopyramide phosphate	RK001-E6
D-118	Phenytoin sodium	RK001-F6
D-119	Danazol	RK001-G6
D-130	DPMA	RK001-H6
D-146	Debrisoquin sulfate	RK001-A7
D-151	P1,P4-Di(adenosine-5')tetraphosphate triammonium	RK001-B7
D-174	Diclofenac sodium	RK001-C7
E-114	erythro-9-(2-Hydroxy-3-nonyl)adenine hydrochloride	RK001-D7
F-122	Furosemide	RK001-E7
I-110	(±)-Ibuprofen	RK001-F7
I-120	Iofetamine hydrochloride	RK001-G7
I-146	IB-MECA	RK001-H7
L-102	Lidocaine hydrochloride	RK001-A8

L-106	Loxapine succinate	RK001-B8
M-101	N6-Methyladenosine	RK001-C8
M-116	Metolazone	RK001-D8
M-128	alpha,beta-Methylene adenosine 5'-triphosphate dilithium	RK001-E8
M-152	2-Methylthioadenosine diphosphate trisodium	RK001-F8
A-202	N6-2-(4-Aminophenyl)ethyladenosine	RK001-H8
M-225	Metrifudil	RK001-G8
N-127	S-(4-Nitrobenzyl)-6-thioinosine	RK001-A9
N-128	S-(4-Nitrobenzyl)-6-thioguanosine	RK001-B9
N-154	N6-Cyclopentyl-9-methyladenine	RK001-C9
P-016	Isoxanthopterin	RK001-D9
A-022	1,3-Dipropyl-8-p-sulfophenylxanthine	RK001-E9
P-101	2-Phenylaminoadenosine	RK001-F9
P-107	N6-2-Phenylethyladenosine	RK001-G9
P-108	N6-Phenyladenosine	RK001-H9
P-121	Phenylbutazone	RK001-A10
C-102	8-Cyclopentyl-1,3-dimethylxanthine	RK001-B5
P-141	Podophyllotoxin	RK001-B10
P-171	Primidone	RK001-C10
P-178	PPADS	RK001-D10
Q-105	Quinine sulfate	RK001-E10
R-102	Reserpine	RK001-F10
S-141	Spironolactone	RK001-G10
S-142	Sulindac	RK001-H10
S-149	Suramin hexasodium	RK001-A11
T-112	Tracazolate	RK001-B11
T-114	Tetracaine hydrochloride	RK001-C11
T-135	Tolazamide	RK001-D11
T-136	Tolbutamide	RK001-E11
V-103	(±)-Vanillylmandelic acid	RK001-F11
V-107	Valproic acid sodium	RK001-G11
X-100	Xanthine amine congener	RK001-H11
A-109	Albuterol hemisulfate	RK002-A2
A-131	Alprenolol hydrochloride	RK002-B2
A-139	(±)-Atenolol	RK002-C2
A-198	Agmatine sulfate	RK002-D2
A-252	AGN 192403 hydrochloride	RK002-E2
B-001	Clonidine hydrochloride	RK002-F2
B-002	p-Aminoclonidine hydrochloride	RK002-G2
B-004	(±)-threo-DOPS	RK002-H2
B-009	DSP-4 hydrochloride	RK002-A3
B-010	Benextramine tetrahydrochloride	RK002-B3
B-011	MHPG sulfate potassium	RK002-C3
B-012	6-Fluoronorepinephrine hydrochloride	RK002-D3
B-013	Xylamine hydrochloride	RK002-E3
B-016	Benoxathian hydrochloride	RK002-F3
B-017	MHPG piperazine	RK002-G3
B-018	WB-4101 hydrochloride	RK002-H3
B-019	Phenoxybenzamine hydrochloride	RK002-A4

B-114	Bretylium tosylate	RK002-B4
B-154	BU224 hydrochloride	RK002-C4
B-161	B-HT 933 dihydrochloride	RK002-D4
C-247	Cyclazosin hydrochloride	RK002-E4
B-169	BRL 37344 sodium	RK002-F4
C-106	CGS-12066A dimaleate	RK002-G4
C-118	Cimetidine	RK002-H4
C-119	(±)-Chlorpheniramine maleate	RK002-A5
C-125	(±)-CGP-12177A hydrochloride	RK002-B5
C-209	Clobenpropit dihydrobromide	RK002-C5
C-223	Cirazoline hydrochloride	RK002-D5
C-231	CGP 20712A methanesulfonate	RK002-E5
D-128	Dimaprit dihydrochloride	RK002-F5
D-158	Diphenhydramine hydrochloride	RK002-G5
D-172	Dobutamine hydrochloride	RK002-H5
I-102	R(-)-Isoproterenol (+)-bitartrate	RK002-B6
E-104	(-)-Epinephrine bitartrate	RK002-A6
G-110	Guanabenz acetate	RK002-D6
H-117	L-Histidine hydrochloride	RK002-E6
H-119	Histamine dihydrochloride	RK002-F6
H-128	Histamine, R(-)-alpha-methyl-, dihydrochloride	RK002-G6
H-136	Histamine, N-alpha-methyl-, dihydrochloride	RK002-H6
H-149	Hydrochlorothiazide	RK002-B7
I-104	(±)-Isoproterenol hydrochloride	RK002-C7
I-114	p-Iodoclonidine hydrochloride	RK002-D7
I-103	S(+)-Isoproterenol (+)-bitartrate	RK002-C6
I-127	ICI 118,551 hydrochloride	RK002-E7
I-135	Imetit dihydrobromide	RK002-F7
A-142	R(+)-Atenolol	RK002-G7
M-133	(-)-alpha-Methylnorepinephrine	RK002-H7
M-134	Methoxamine hydrochloride	RK002-A8
N-111	(±)-Normetanephrine hydrochloride	RK002-B8
N-113	L(-)-Norepinephrine bitartrate	RK002-C8
A-143	S(-)-Atenolol	RK002-D8
N-151	Nisoxetine hydrochloride	RK002-E8
N-153	Nylidrin hydrochloride	RK002-F8
N-158	Naftopidil dihydrochloride	RK002-G8
O-101	(±)-Octopamine hydrochloride	RK002-H8
O-110	Oxymetazoline hydrochloride	RK002-A9
P-115	Prazosin hydrochloride	RK002-B9
P-119	(±)-Pindobind	RK002-C9
P-124	Prazobind	RK002-D9
P-125	Pindolol	RK002-E9
P-128	(±)-Propranolol hydrochloride	RK002-F9
P-129	Pyrilamine maleate	RK002-G9
P-131	Phentolamine mesylate	RK002-H9
P-133	Phenylephrine hydrochloride	RK002-A10
U-101	Urapidil, 5-Methyl-	RK002-B10
P-169	Protriptyline hydrochloride	RK002-C10

P-172	Promethazine hydrochloride	RK002-D10
R-101	Ranitidine hydrochloride	RK002-E10
R-104	Rauwolscine hydrochloride	RK002-F10
H-137	Histamine, 1-methyl-, dihydrochloride	RK002-A7
S-145	SKF 91488 dihydrochloride	RK002-G10
T-118	Triprolidine hydrochloride	RK002-H10
T-123	Thioperamide maleate	RK002-A11
T-141	Tripelennamine hydrochloride	RK002-B11
T-148	S(-)-Timolol maleate	RK002-C11
U-100	Urapidil hydrochloride	RK002-D11
U-104	UK 14,304	RK002-E11
X-101	Xylazine hydrochloride	RK002-F11
Y-100	Yohimbine hydrochloride	RK002-G11
Y-101	YS-035 hydrochloride	RK002-H11
A-104	N-Aminodeanol chloride	RK003-A2
A-105	Atropine sulfate	RK003-B2
A-113	Amiloride hydrochloride	RK003-C2
A-119	Amiodarone hydrochloride	RK003-D2
A-134	4-Aminopyridine	RK003-E2
A-138	Aminobenztropine	RK003-F2
A-140	Arecaidine propargyl ester hydrobromide	RK003-G2
A-148	N-Acetylprocainamide hydrochloride	RK003-H2
A-185	Ambenonium dichloride	RK003-A3
A-251	A-85380 dihydrochloride	RK003-B3
B-105	Bethanechol chloride	RK003-C3
B-108	Benztropine mesylate	RK003-D3
B-111	Bepridil hydrochloride	RK003-E3
B-112	(±)-Bay K 8644	RK003-F3
B-149	2,3-Butanedione monoxime	RK003-G3
C-006	Arecoline hydrobromide	RK003-H3
C-007	10-(alpha-Diethylaminopropionyl)-phenothiazine	RK003-A4
C-008	(+)-cis-Dioxolane	RK003-B4
C-009	McN-A-343	RK003-C4
C-011	OXA-22	RK003-D4
C-107	Carbachol	RK003-E4
D-104	4-DAMP methiodide	RK003-G4
D-112	Diltiazem hydrochloride	RK003-H4
D-129	R(+)-Butylindazone	RK003-A5
D-136	Diazoxide	RK003-B5
D-149	Dihydro-beta-erythroidine hydrobromide	RK003-C5
D-189	1,1-Dimethyl-4-phenyl-piperazinium iodide	RK003-D5
D-212	Decamethonium dibromide	RK003-E5
E-100	(-)-Eseroline fumarate	RK003-F5
F-107	Flunarizine dihydrochloride	RK003-G5
F-131	FPL 64176	RK003-H5
G-104	Gallamine triethiodide	RK003-A6
G-106	Glibenclamide	RK003-B6
G-117	Glipizide	RK003-C6
H-126	Hexahydro-sila-difenidol hydrochloride	RK003-D6

H-127	Hexahydro-sila-difenidol hydrochloride, p-fluoro analog	RK003-E6
H-132	Hexamethonium dichloride	RK003-F6
H-172	(+)-Himbacine	RK003-G6
I-108	Ipratropium bromide	RK003-H6
I-117	R(+)-IAA-94	RK003-A7
L-108	Lidocaine, N-ethyl bromide quaternarysalt	RK003-B7
L-134	Linopirdine	RK003-C7
C-258	CDD 0097 hydrochloride	RK003-F4
M-105	Methoctramine tetrahydrochloride	RK003-D7
M-106	Mecamylamine hydrochloride	RK003-E7
M-115	(±)-Methoxy verapamil hydrochloride	RK003-F7
M-140	Methyl carbamylcholine chloride	RK003-G7
M-142	Minoxidil	RK003-H7
M-169	Methyl furtrethonium iodide	RK003-A8
N-110	Neostigmine bromide	RK003-B8
N-114	Nifedipine	RK003-C8
N-126	Nicardipine hydrochloride	RK003-D8
N-134	(±)-Niguldipine hydrochloride	RK003-E8
N-144	Nitrendipine	RK003-F8
N-149	Nimodipine	RK003-G8
N-150	5-Nitro-2-(3-phenylpropylamino) benzoic acid	RK003-H8
N-170	NS-1619	RK003-A9
O-100	Oxotremorine methiodide	RK003-B9
P-113	(+)-Pilocarpine hydrochloride	RK003-C9
P-114	Pirenzepine dihydrochloride	RK003-D9
P-137	Procainamide hydrochloride	RK003-E9
P-142	Pyridostigmine bromide	RK003-F9
P-149	Pralidoxime iodide	RK003-G9
P-154	Pinacidil	RK003-H9
P-155	(-)-Physostigmine	RK003-A10
P-160	N-Phenylanthranilic acid	RK003-B10
P-203	Phenamil methanesulfonate	RK003-C10
Q-106	Quinidine sulfate	RK003-D10
S-104	(-)-Scopolamine hydrobromide	RK003-E10
S-105	(-)-Scopolamine,n-Butyl-, bromide	RK003-F10
S-117	Succinylcholine chloride	RK003-G10
T-105	Tetraethylammonium chloride	RK003-H10
T-111	TMB-8 hydrochloride	RK003-A11
T-122	Telenzepine dihydrochloride	RK003-B11
T-125	Trihexyphenidyl hydrochloride	RK003-C11
T-139	Triamterene	RK003-D11
T-142	Taxol	RK003-E11
T-167	Tropicamide	RK003-F11
V-100	(±)-Vesamicol hydrochloride	RK003-G11
V-102	(±)-Verapamil hydrochloride	RK003-H11
A-132	Amantadine hydrochloride	RK004-A2
A-206	Agroclavine	RK004-B2
A-255	A-77636 hydrochloride	RK004-C2
B-102	Bupropion hydrochloride	RK004-D2

B-115	(+)-Bromocriptine methanesulfonate	RK004-E2
B-122	Benserazide hydrochloride	RK004-F2
B-135	R(+)-6-Bromo-APB hydrobromide	RK004-G2
B-168	(±)-Butaclamol hydrochloride	RK004-H2
C-126	S-(-)-Carbidopa	RK004-A3
C-130	(±)-Chloro-APB hydrobromide	RK004-B3
C-134	Chlorpromazine hydrochloride	RK004-C3
C-171	Clozapine	RK004-D3
C-207	4'-Chloro-3-alpha-(diphenylmethoxy)tropane hydrochloride	RK004-E3
D-002	6,7-ADTN hydrobromide	RK004-F3
D-003	R(-)-Apocodeine hydrochloride	RK004-G3
D-004	R(-)-Apomorphine hydrochloride	RK004-H3
D-016	4-Hydroxy-3-methoxyphenylacetic acid	RK004-A4
D-018	4-Hydroxyphenethylamine hydrochloride	RK004-B4
D-019	Dopamine hydrochloride	RK004-C4
D-020	3-Methoxy-4-hydroxyphenethylamine hydrochloride	RK004-D4
D-021	4-Methoxy-3-hydroxyphenethylamine	RK004-E4
D-022	N-Methyldopamine hydrochloride	RK004-F4
D-027	R(-)-Propylnorapomorphine hydrochloride	RK004-G4
D-029	R(-)-2,10,11-Trihydroxyaporphine hybrobromide	RK004-H4
D-030	R(-)-2,10,11-Trihydroxy-N-propylnoraporphine	RK004-A5
D-031	Dipropyldopamine hydrobromide	RK004-B5
D-040	R(-)-Norapomorphine hydrobromide	RK004-C5
D-042	R(-)-N-Allyl norapomorphine hydrobromide	RK004-D5
D-044	Amfonelic acid	RK004-E5
D-046	(+)-Bulbocapnine hydrochloride	RK004-F5
D-047	(±)-SKF-38393 hydrochloride	RK004-G5
D-050	Spiperone hydrochloride	RK004-H5
D-052	GBR-12909 dihydrochloride	RK004-A6
D-054	R(+)-SCH-23390 hydrochloride	RK004-B6
D-122	Domperidone	RK004-C6
D-155	Dihydroergocristine methanesulfonate	RK004-D6
D-156	Dihydroergotamine methanesulfonate	RK004-E6
D-008	R(-)-2,11-Dihydroxy-10-methoxyaporphine hydrochloride	RK004-F6
D-206	S(-)-DS 121 hydrochloride	RK004-G6
E-140	Ergocristine	RK004-H6
D-009	L-3,4-Dihydroxyphenylalanine	RK004-A7
F-100	Fluspirilene	RK004-B7
F-101	Fluphenazine dihydrochloride	RK004-C7
F-114	cis(Z)-Flupentixol dihydrochloride	RK004-D7
H-100	Haloperidol	RK004-E7
H-145	(±)-7-Hydroxy-DPAT hydrobromide	RK004-G7
I-119	Indatraline hydrochloride	RK004-H7
J-102	JL-18	RK004-A8
L-118	R(+)-Lisuride hydrogen maleate	RK004-B8
L-131	L-745,870 hydrochloride	RK004-C8
M-117	Metoclopramide hydrochloride	RK004-D8
M-153	Mesulergine hydrochloride	RK004-E8
N-123	Nomifensine maleate	RK004-F8

O-111	(±)-Octoclothepin maleate	RK004-G8
P-037	(6R)-5,6,7,8-Tetrahydro-L-biopterin hydrochloride	RK004-H8
P-100	Pimozide	RK004-A9
P-102	R(+)-3PPP hydrochloride	RK004-B9
P-105	(±)-PPHT hydrochloride	RK004-D9
P-122	Prochlorperazine dimaleate	RK004-E9
P-168	Pergolide methanesulfonate	RK004-F9
P-183	S(+)-PD 128,907 hydrochloride	RK004-G9
Q-102	(-)-Quinpirole hydrochloride	RK004-H9
Q-110	Quinelorane dihydrochloride	RK004-A10
R-118	Risperidone	RK004-B10
R-121	S(-)-Raclopride L-tartrate	RK004-C10
R-123	RBI-257 maleate	RK004-D10
S-116	(±)-Sulpiride	RK004-E10
S-143	(±)-6-Chloro-PB hydrobromide	RK004-F10
S-159	R(-)-SCH-12679 maleate	RK004-G10
S-168	(±)-SKF 38393, N-allyl-, hydrobromide	RK004-H10
G-120	GYKI 52895	RK004-A11
H-109	3-Hydroxybenzylhydrazine dihydrochloride	RK004-F7
P-103	S(-)-3PPP hydrochloride	RK004-C9
T-103	Trifluperidol hydrochloride	RK004-B11
T-106	Thiothixene hydrochloride	RK004-C11
T-107	Trifluoperazine dihydrochloride	RK004-D11
T-108	Thioridazine hydrochloride	RK004-E11
T-165	R(+)-Terguride	RK004-F11
U-115	U-101958 maleate	RK004-G11
U-116	U-99194A maleate	RK004-H11
A-102	(±)-2-Amino-4-phosphonobutyric acid	RK005-A2
A-103	L-Aspartic acid	RK005-B2
A-108	2-Amino-3-phosphonopropionic acid	RK005-C2
A-110	2-Amino-5-phosphonopentanoic acid	RK005-D2
A-126	(±)-HA-966	RK005-E2
A-263	ATPA	RK005-F2
A-155	trans-(±)-ACPD	RK005-G2
A-156	(±)-N-Allylnormetazocine hydrochloride	RK005-H2
A-158	Arcaine sulfate	RK005-A3
A-162	1-Amino-1-cyclohexanecarboxylic acid hydrochloride	RK005-B3
A-163	Aniracetam	RK005-C3
A-172	(±)-1-Aminocyclobutane-cis-1,3-dicarboxylic acid	RK005-D3
B-171	1-BCP	RK005-E3
A-243	cis-Azetidine-2,4-dicarboxylic acid	RK005-F3
A-244	trans-Azetidine-2,4-dicarboxylic acid	RK005-G3
A-254	AIDA	RK005-H3
C-104	(±)-CPP	RK005-A4
C-121	7-Chlorokynurenic acid	RK005-B4
C-124	beta-CFT naphthalene sulfonate	RK005-C4
C-271	CX 546	RK005-D4
C-137	L-Cysteine hydrochloride	RK005-E4
C-146	D-Cycloserine	RK005-F4

C-147	(+)-Cyclazocine	RK005-G4
C-161	Calcimycin	RK005-H4
C-163	Carbetapentane citrate	RK005-A5
C-170	R(-)PPAP hydrochloride	RK005-B5
C-191	Capsazepine	RK005-C5
F-154	Felbamate	RK005-D5
C-203	2-Chloro-2-deoxy-D-glucose	RK005-E5
C-212	L-Cysteine, N-Acetyl-	RK005-F5
C-215	S(+)-4-Carboxyphenylglycine	RK005-G5
C-239	CNQX disodium	RK005-H5
D-115	Dextromethorphan hydrobromide	RK005-A6
D-123	DNQX	RK005-B6
D-127	Dextrorphan D-tartrate	RK005-C6
D-133	6,7-Dichloroquinoxaline-2,3-dione	RK005-D6
D-138	5,7-Dichlorokynurenic acid	RK005-E6
D-140	1,10-Diaminodecane	RK005-F6
F-109	5-Fluoroindole-2-carboxylic acid	RK005-G6
G-017	AMPA hydrobromide	RK005-H6
G-018	(±)-2-Amino-7-phosphonoheptanoic acid	RK005-A7
G-020	Kainic acid	RK005-B7
G-100	L-Glutamic acid hydrochloride	RK005-C7
G-107	L-Glutamine	RK005-D7
G-111	D-gamma-Glutamylaminomethanesulfonic acid	RK005-E7
G-118	L-Glutamic acid, N-phthaloyl-	RK005-F7
G-119	GYKI 52466 hydrochloride	RK005-G7
G-137	(2S,4R)-4-Methylglutamic acid	RK005-H7
I-118	Ifenprodil tartrate	RK005-C8
I-139	S(-)-IBZM	RK005-D8
K-100	Kynurenic acid	RK005-E8
M-102	N-Methyl-D-aspartic acid	RK005-F8
M-107	(+)-MK-801 hydrogen maleate	RK005-G8
M-166	MDL 26,630 trihydrochloride	RK005-H8
M-172	(±)-alpha-Methyl-4-carboxyphenylglycine	RK005-A9
M-183	Memantine hydrochloride	RK005-B9
M-187	3-Methoxy-morphanin hydrochloride	RK005-C9
M-216	MDL 105,519	RK005-D9
N-138	(+)-Normetazocine	RK005-E9
N-179	NS 102	RK005-F9
H-174	(±)-3-Hydroxy-phenylglycine	RK005-A8
I-116	(±)-Ibotenic acid	RK005-B8
N-183	NBQX disodium	RK005-G9
P-111	(±)-cis-Piperidine-2,3-dicarboxylic acid	RK005-H9
P-156	O-Phospho-L-serine	RK005-A10
P-161	Pentamidine isethionate	RK005-B10
P-167	L-trans-Pyrollidine-2,4-dicarboxylic acid	RK005-C10
P-185	Propofol	RK005-D10
P-204	Phenylbenzene-omega-phosphono-alpha-amino acid	RK005-E10
P-209	Phthalamoyl-L-glutamic acid trisodium	RK005-F10
Q-103	(+)-Quisqualic acid	RK005-G10

Q-104	Quinolinic acid	RK005-H10
R-112	Rimcazole dihydrochloride	RK005-A11
R-116	Riluzole	RK005-B11
S-126	Spermine tetrahydrochloride	RK005-C11
S-135	D-Serine	RK005-D11
U-106	(-)-cis-(1S,2R)-U-50488 tartrate	RK005-E11
U-113	Hydrouracil, (±)-cis-5-fluoro-6-hydroxy-	RK005-F11
U-114	Uracil, (±)-5-trifluoromethyl-5,6-dihydro-	RK005-G11
W-105	S(-)-Willardiine	RK005-H11
A-160	NG-Nitro-L-arginine	RK006-A2
A-161	NG-Nitro-L-arginine methyl ester hydrochloride	RK006-B2
A-165	Rp-cAMPS triethylamine	RK006-C2
A-166	Sp-cAMPS triethylamine	RK006-D2
A-199	Aminoguanidine hemisulfate	RK006-E2
A-200	L-Arginine	RK006-F2
A-222	AG 1478	RK006-G2
A-223	AG 1295	RK006-H2
A-253	AMT hydrochloride	RK006-A3
B-109	nor-Binaltorphimine dihydrochloride	RK006-B3
B-125	(±)-Bremazocine hydrochloride	RK006-C3
B-130	8-Bromo-cAMP sodium	RK006-D3
B-131	8-Bromo-cGMP sodium	RK006-E3
B-170	BW373U86 hydrochloride	RK006-F3
C-100	Calmidazolium chloride	RK006-G3
C-165	8-(4-Chlorophenylthio)-cAMP sodium	RK006-H3
C-186	Ceramide	RK006-A4
C-198	Chelerythrine chloride	RK006-B4
C-221	Carboxy-PTIO potassium	RK006-C4
C-224	Cyclosporin A	RK006-D4
D-145	Dantrolene sodium	RK006-E4
D-175	5,5-Dimethyl-1-pyrroline-N-oxide	RK006-F4
D-176	Daidzein	RK006-G4
D-183	2,4-Diamino-6-hydroxypyrimidine	RK006-H4
D-186	D609 potassium	RK006-A5
D-198	Diphenyleneiodonium chloride	RK006-B5
D-210	4,5-Dianilinophthalimide	RK006-C5
E-120	Erbstatin analog	RK006-D5
F-105	Forskolin	RK006-E5
G-103	Genistein	RK006-F5
I-159	Isoliquiritigenin	RK006-G5
G-133	GR-89696 fumarate	RK006-H5
H-120	HA-1004 hydrochloride	RK006-A6
H-121	H-7 dihydrochloride	RK006-B6
H-122	H-8 dihydrochloride	RK006-C6
H-123	H-9 dihydrochloride	RK006-D6
I-122	ICI 204,448 hydrochloride	RK006-E6
I-134	L-N5-(1-Iminoethyl)-ornithine hydrochloride	RK006-F6
I-150	L- N6-(1-Iminoethyl)lysine hydrochloride	RK006-G6
K-102	KN-62	RK006-H6

L-104	Loperamide hydrochloride	RK006-A7
L-121	Levallorphan tartrate	RK006-B7
L-132	LY-294,002 hydrochloride	RK006-C7
M-125	NG-Monomethyl-L-arginine acetate	RK006-D7
M-182	MDL 12,330A hydrochloride	RK006-E7
M-224	S-Methylisothiourea hemisulfate	RK006-F7
N-119	Noscapine	RK006-A8
N-120	(-)-Norcodeine	RK006-B8
N-122	Naloxonazine	RK006-C8
N-156	Naltriben methanesulfonate	RK006-D8
N-161	NPC-15437 dihydrochloride	RK006-E8
N-163	7-Nitroindazole	RK006-F8
N-165	Naloxone benzoylhydrazone	RK006-G8
N-182	NADPH tetrasodium	RK006-H8
O-002	Naloxone hydrochloride	RK006-B9
O-004	Naltrexone hydrochloride	RK006-C9
O-005	Nalbuphine hydrochloride	RK006-D9
O-120	Olomoucine	RK006-E9
O-121	ODQ	RK006-F9
P-215	PD 098,059	RK006-G9
S-122	SC-10	RK006-H9
S-146	Sphingosine	RK006-A10
S-153	SQ 22536	RK006-B10
S-154	Sepiapterin	RK006-C10
T-126	Tamoxifen citrate	RK006-D10
M-270	Milrinone	RK006-E10
T-171	Tamoxifen, 3-hydroxy-, citrate, (E)-	RK006-F10
T-173	Thiocitrulline	RK006-G10
T-175	1-(-2-Trifluoromethylphenyl)imidazole	RK006-H10
T-177	Thio-NADP sodium	RK006-A11
M-231	(-)-3-Methoxynaltrexone hydrochloride	RK006-G7
N-115	Naltrindole hydrochloride	RK006-H7
U-102	(±) trans-U-50488 methanesulfonate	RK006-C11
U-105	U-62066	RK006-D11
U-107	U-73122	RK006-E11
U-112	U-73343	RK006-F11
W-101	W-7 hydrochloride	RK006-G11
N-211	NS 2028	RK006-A9
T-182	Tyrphostin A9	RK006-B11
Z-104	Zaprinast	RK006-H11
A-117	Amitriptyline hydrochloride	RK007-A2
A-118	Azathioprine	RK007-B2
A-129	Amoxapine	RK007-C2
A-164	Alaproclate hydrochloride	RK007-D2
B-173	BRL 54443 maleate	RK007-E2
A-178	Acetohexamide	RK007-F2
A-250	Acyclovir	RK007-G2
B-119	Buspirone hydrochloride	RK007-H2
B-134	BMY 7378 dihydrochloride	RK007-A3

C-111	(±)-p-Chlorophenylalanine	RK007-B3
C-112	Cyproheptadine hydrochloride	RK007-C3
C-113	Carisoprodol	RK007-D3
C-114	Carbamazepine	RK007-E3
C-115	Clofibrate	RK007-F3
C-117	5-Carboxamidotryptamine maleate	RK007-G3
C-128	Clofilium tosylate	RK007-H3
C-129	Clomipramine hydrochloride	RK007-A4
C-138	Cysteamine hydrochloride	RK007-B4
C-144	1-(m-Chlorophenyl)-biguanide hydrochloride	RK007-C4
C-177	Chloroquine phosphate	RK007-D4
C-181	Cinanserin	RK007-E4
C-242	CI-988 N-methyl-D-glucamine	RK007-F4
D-101	(±)-DOI hydrochloride	RK007-G4
D-124	Doxepin hydrochloride	RK007-H4
D-125	Desipramine hydrochloride	RK007-A5
D-148	5,7-Dihydroxytryptamine creatinine sulfate	RK007-B5
F-132	Fluoxetine hydrochloride	RK007-C5
H-115	5-Hydroxy-L-tryptophan	RK007-F5
I-101	Iproniazid phosphate	RK007-G5
I-111	Imipramine hydrochloride	RK007-H5
L-107	LY-53,857 maleate	RK007-A6
L-109	Lorglumide sodium	RK007-B6
L-110	LY-278,584 maleate	RK007-C6
L-119	L-703,606 oxalate	RK007-D6
M-001	Proglumide	RK007-E6
M-007	Benzotript	RK007-F6
M-109	2-Methyl-5-hydroxytryptamine maleate	RK007-G6
M-110	alpha-Methyl-5-hydroxytryptamine maleate	RK007-H6
M-113	Melatonin	RK007-A7
M-149	Methiothepin mesylate	RK007-B7
M-167	Metergoline	RK007-C7
M-204	p-MPPI hydrochloride	RK007-D7
N-116	Nortriptyline hydrochloride	RK007-E7
N-124	NAN-190 hydrobromide	RK007-F7
N-178	5-(Nonyloxy)-tryptamine hydrogen oxalate	RK007-G7
P-120	1-Phenylbiguanide	RK007-H7
P-126	Pirenperone	RK007-A8
P-150	Pindobind-5HT1A	RK007-B8
P-162	Pregnenolone sulfate sodium	RK007-C8
W-108	WAY-100635 maleate	RK007-G11
P-218	PD 142,898 N-methyl-D-glucamine	RK007-D8
Q-107	Quipazine, N-methyl-, dimaleate	RK007-E8
Q-109	Quipazine, 6-nitro-, maleate	RK007-F8
R-103	Ritanserin	RK007-G8
S-002	(±)-8-Hydroxy-DPAT hydrobromide	RK007-H8
S-003	1-(1-Naphthyl)piperazine hydrochloride	RK007-A9
S-004	5-Methoxy DMT oxalate	RK007-B9
S-005	TFMPP hydrochloride	RK007-C9

S-006	Ketanserin tartrate	RK007-D9
S-007	Quipazine dimaleate	RK007-E9
S-008	1-(2-Methoxyphenyl)piperazine hydrochloride	RK007-F9
S-009	PAPP	RK007-G9
S-011	Serotonin hydrochloride	RK007-H9
S-014	1-(3-Chlorophenyl)piperazine dihydrochloride	RK007-A10
S-100	Mianserin hydrochloride	RK007-B10
S-103	Spiroxatrine	RK007-C10
S-144	SQ 29,548	RK007-D10
S-174	SDZ-205,557 hydrochloride	RK007-E10
F-133	Norfluoxetine hydrochloride	RK007-D5
H-110	5-Hydroxyindolacetic acid	RK007-E5
S-180	SB 206553 hydrochloride	RK007-F10
T-102	3-Tropanyl-3,5-dichlorobenzoate	RK007-G10
T-104	3-Tropanyl-indole-3-carboxylate hydrochloride	RK007-H10
T-109	Tryptamine hydrochloride	RK007-A11
T-117	L-Tryptophan	RK007-B11
T-137	Trazodone hydrochloride	RK007-C11
U-108	S(-)-UH-301 hydrochloride	RK007-D11
U-109	R(+)-UH-301 hydrochloride	RK007-E11
W-103	WIN 51,708	RK007-F11
Z-101	Zimelidine dihydrochloride	RK007-H11
A-100	9-Amino-1,2,3,4-tetrahydroacridine hydrochloride	RK008-A2
A-120	5-Aminovaleric acid hydrochloride	RK008-B2
A-121	Allopurinol	RK008-C2
A-122	(±)-p-Aminoglutethimide	RK008-D2
A-173	3'-Azido-3'-deoxythymidine	RK008-E2
A-177	Acetazolamide	RK008-F2
A-196	3-Aminopropyl-(methyl)phosphinic acid hydrochloride	RK008-G2
A-201	cis-4-Aminocrotonic acid	RK008-H2
A-230	gamma-Acetylinic GABA	RK008-A3
B-020	(±)-Baclofen	RK008-B3
B-100	S(-)-p-Bromotetramisole oxalate	RK008-C3
B-103	(-)-Bicuculline methbromide, 1(S), 9(R)	RK008-D3
B-145	Benzamide	RK008-E3
C-108	2-Cyclooctyl-2-hydroxyethylamine hydrochloride	RK008-F3
C-140	4'-Chlorodiazepam	RK008-G3
C-157	Captopril	RK008-H3
C-178	Chlorothiazide	RK008-A4
D-023	DL-alpha-Methyl-p-tyrosine	RK008-B4
D-025	Papaverine hydrochloride	RK008-C4
D-026	Pargyline hydrochloride	RK008-D4
D-038	3-Iodo-L-tyrosine	RK008-E4
D-039	L-alpha-Methyl-p-tyrosine	RK008-F4
D-102	Diacylglycerol kinase inhibitor I	RK008-G4
D-103	(±)-2,3-Dichloro-alpha-methylbenzylamine hydrochloride	RK008-H4
D-131	3,5-Dinitrocatechol	RK008-A5
D-193	DL-alpha-Difluoromethylornithine hydrochloride	RK008-B5
E-150	(±)-Etomoxir sodium	RK008-E5

F-123	Fusaric acid	RK008-F5
F-129	FGIN I-27	RK008-G5
G-002	Isoguvacine hydrochloride	RK008-H5
G-005	(±)-Nipecotic acid	RK008-A6
G-006	4,5,6,7-Tetrahydroisoxazolo[4,5-c]pyridin-3-ol	RK008-B6
G-007	Guvacine hydrochloride	RK008-C6
G-008	Thiomuscimol hydrobromide	RK008-D6
G-009	Piperidine-4-sulphonic acid	RK008-E6
G-011	Isonipecotic acid	RK008-F6
G-012	GABA	RK008-G6
G-019	Muscimol hydrobromide	RK008-H6
H-106	Hydroxylamine hydrochloride	RK008-A7
H-108	Hemicholinium-3	RK008-B7
H-113	2-Hydroxysaclofen	RK008-C7
H-138	(+)-Hydrastine	RK008-D7
T-200	TPMPA	RK008-E7
I-109	Indomethacin	RK008-F7
I-138	1,5-Isoquinolinediol	RK008-G7
K-104	Kojic amine hydrobromide	RK008-H7
K-105	Ketoconazole	RK008-A8
M-002	SKF-525A hydrochloride	RK008-B8
M-003	R(-)-Deprenyl hydrochloride	RK008-C8
M-004	Clorgyline hydrochloride	RK008-D8
M-008	6-Methoxy-1,2,3,4-tetrahydro-9H-pyrido[3,4b] indole	RK008-E8
M-129	L-alpha-Methyl DOPA	RK008-F8
E-006	N-Methyl-beta-carboline-3-carboxamide	RK008-C5
E-128	Etazolate hydrochloride	RK008-D5
R-107	Ro 41-1049 hydrochloride MAO-A inhibitor	RK008-G8
N-108	Nialamide	RK008-H8
N-142	NO-711 hydrochloride	RK008-A9
P-001	Aminopterin	RK008-B9
P-106	3-Phenylpropargylamine hydrochloride	RK008-C9
P-109	Tranylcypromine hydrochloride	RK008-D9
P-117	Picrotoxin	RK008-E9
P-118	Phaclofen	RK008-F9
P-130	PK 11195	RK008-G9
P-179	Pentoxifylline	RK008-H9
P-205	Propentofylline	RK008-A10
Q-100	Quinacrine dihydrochloride	RK008-B10
R-106	Ro 16-6491 hydrochloride	RK008-C10
R-108	Ro 41-0960	RK008-D10
R-109	Ro 15-4513	RK008-E10
R-110	Ro 05-3663	RK008-F10
R-111	Ro 20-1724	RK008-G10
R-122	Rolipram	RK008-H10
S-106	SR-95531	RK008-A11
S-107	Semicarbazide hydrochloride	RK008-B11
S-147	Sulfaphenazole	RK008-C11
S-148	Sanguinarine chloride	RK008-D11

F-124	Furafylline	RK008-E11
T-101	THIP hydrochloride	RK008-F11
T-140	Trimethoprim	RK008-G11
V-110	(±)-gamma-Vinyl GABA	RK008-H11

ADENOSINE, PURINERGIC$_1$, A$_1$
BINDING ASSAY
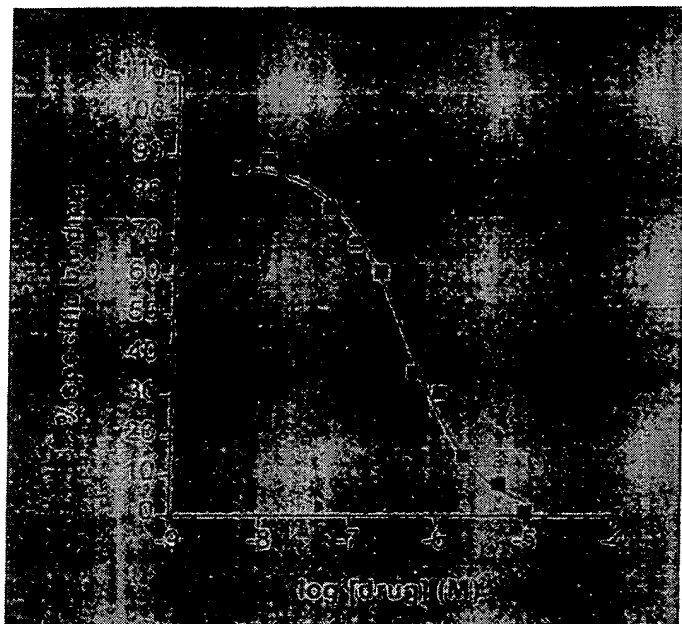
Reference Compounds	Ki (nM)
CPA	5.3
CHA	18.1
NECA	59.1
■ 2-CADO	117.0
MECA	221.0

Assay Characteristics:

K$_D$ (binding affinity):	0.97 nM
B$_{max}$ (receptor number):	46 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]-8-Cyclopentyl-1,3-dipropylxanthine (CPX) (80-120 Ci/mmol)
	Final ligand concentration - [0.8 nM]
Non-specific Determinant:	2-Chloroadenosine (CADO) - [10 μM]
Reference Compound:	2-Chloroadenosine (CADO)
Positive Control:	2-Chloroadenosine (CADO)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the adenosine$_1$ binding site.

Literature Reference:

Bruns, R., Fergus, R., Badger, E., Bristol, J., Santay, et al. Binding of the A$_1$-Selective Adenosine Antagonist 8-Cyclopentyl-1,3-Dipropylxanthine to Rat Brain Membranes. *Naunyn-Schmeideberg Archives of Pharmacology.* **335**: 59-63 (1987) with modifications.

Ferkany, J.W., Valentine, H.L., Stone, G.W., and Williams, M. Adenosine A$_1$ Receptors in Mammalian Brain: Species Differences with Agonists and Antagonists. *Drug Dev. Res.* **9**: 85-93 (1986).

ADENOSINE, PURINERGIC$_1$, NON-SELECTIVE
BINDING ASSAY
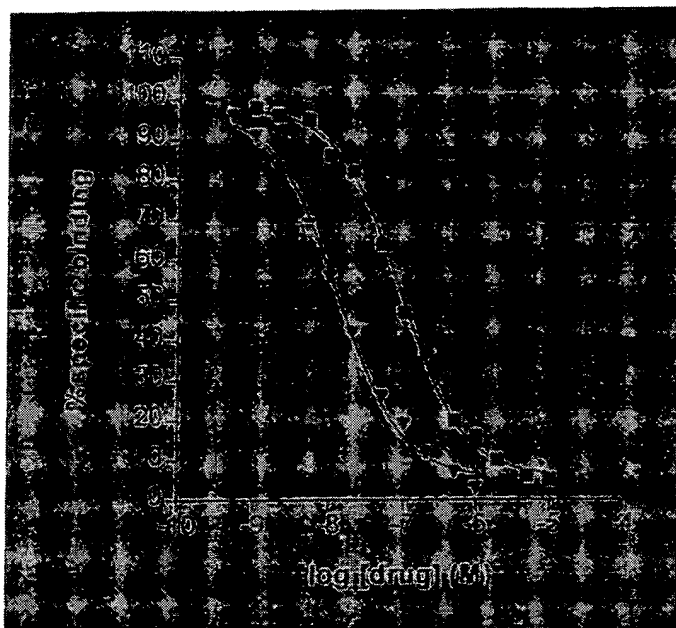
Reference Compounds	Ki (nM)
▼ NECA	8.5
■ MECA	77.0

Assay Characteristics:

K$_D$ (binding affinity):   7.7 nM

B$_{max}$ (receptor number):   11.4 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Bovine striatal membranes

Radioligand: [^{3}H] 5'-N-ethylcarboxamidoadenosine (NECA) (15-30 Ci/mmol)
Final ligand concentration - [4.0 nM]

Non-specific Determinant: NECA (5'-N-Ethylcarboxamidoadenosine) - [10 µM]

Reference Compound: MECA (5'-N-Methylcarboxamidoadenosine)

Positive Control: MECA (5'-N-Methylcarboxamidoadenosine)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the adenosine binding site.

Literature Reference: Bruns, R., Lu, G. and Pugsley, T. Characterization of the A$_2$ Adenosine Receptor Labeled by [^{3}H]-NECA in Rat Striatal Membrane. *Pharmacology.* **29**: 331-346 (1986) with modifications.

Weir, R. L., Anderson, S. M., et al.   Inhibition of N6-[^{3}H]-CHA Binding by Carbamazepine.   *Epilepsia.* **31(5)**: 503-512 (1990) with modifications.

Holtzman, S. G., et al. Role of Adenosine Receptors in Caffeine Tolerance. *Jrnl. Pharm. & Exp. Ther.* **256(1)**: 62-67 (1990).

ADENOSINE, PURINERGIC₁, A₂
BINDING ASSAY
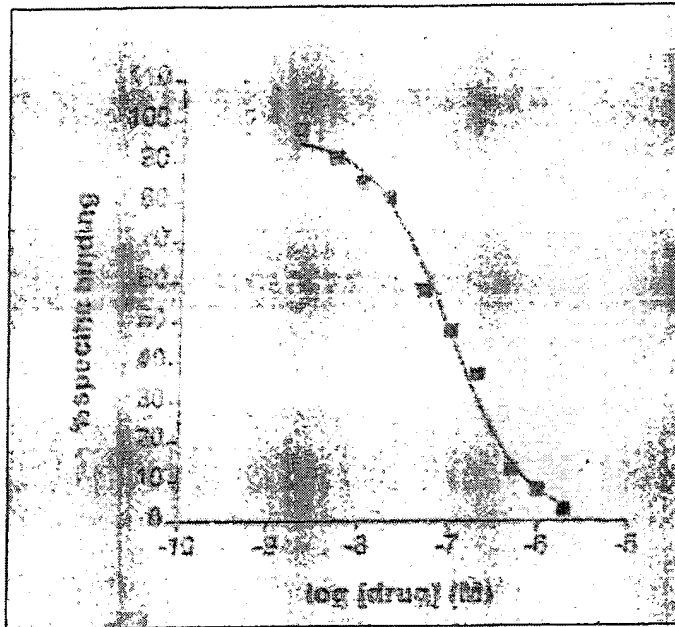
Reference Compounds	Ki (nM)
□ 2-CADO	46.0
MECA	202.7
CPX	550.0
CPA	566.3
CHA	775.0

Assay Characteristics:

K_D (binding affinity):   6.0 nM

B_{max} (receptor number):   21 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Bovine striatal membranes

Radioligand: [³H]CGS 21680 (30-60 Ci/mmol)
Final ligand concentration - [5.0 nM]

Non-specific Determinant: 2-Chloroadenosine (2-CADO) - [10.0 µM]

Reference Compound: 2-Chloroadenosine (2-CADO)

Positive Control: 2-Chloroadenosine (2-CADO)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) 10 mM $MgCl_2$ for 90 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the adenosine₂ binding site.

Literature Reference:

M. Jarvis, R. Schulz, A. Hutchison, U. Do, M. Sills, and M. Williams. [³H]CGS 21680: A Selective Adenosine 2 Receptor Agonist Directly Labels Adenosine 2 Receptors in Rat Brain. *Jrnl. Pharmacol. and Exper. Therap.* **251(3):** 888-893 (1989) with modifications.

Bruns, R.F., Lu, G.H., and Pugsley, T.A. Characterization of the A₂ Adenosine Receptor Labeled by [³H[NECA in Rat Striata Membranes. *Mol. Pharmacol.* **29:** 331-346 (1986) with modifications.

ADENOSINE, PURINERGIC, A$_1$ (HUMAN RECOMBINANT)
BINDING ASSAY
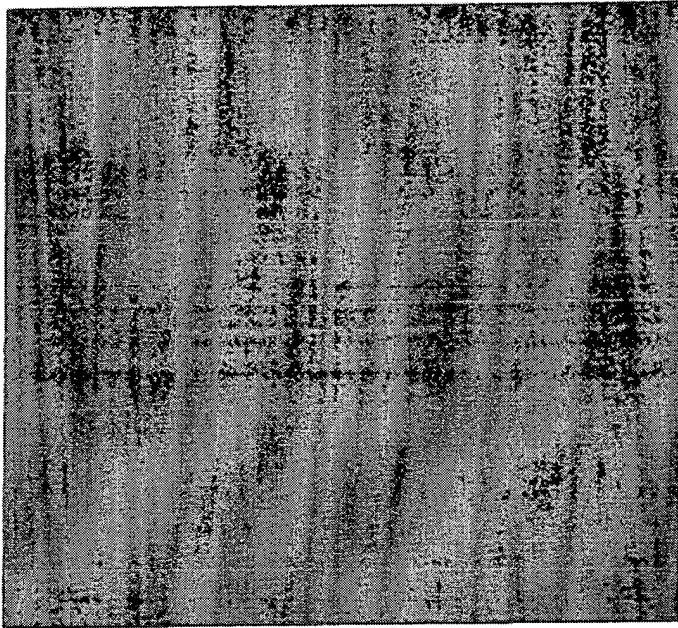
ADENOSINE, PURINERGIC$_1$, A$_3$ (HUMAN RECOMBINANT)
BINDING ASSAY
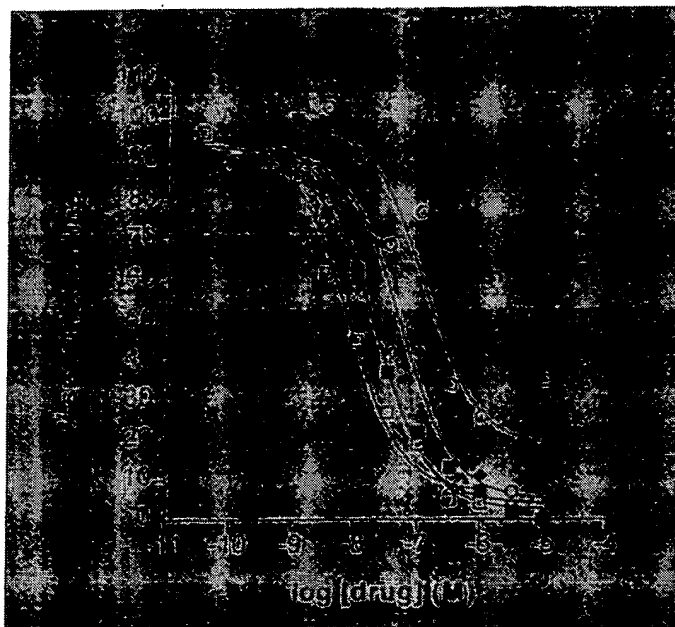
Reference Compounds	Ki (nM)
□ AB-MECA	5.6
■ NECA	13.7
× MECA	16.5
◆ 2-CADO	37.9
○ PIA	86.2

Assay Characteristics:

K$_D$ (binding affinity):	1.5 nM
B$_{max}$ (receptor number):	0.3 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in HEK 293 cells
Radioligand:	[^{125}I]AB-MECA (2000 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specific Determinant:	NECA - [10.0 µM]
Reference Compound:	NECA
Positive Control:	NECA
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 8.0) containing 10 mM MgCl$_2$, 1 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned adenosine$_3$ binding site.

Literature Reference:	Salvatore, C.A., et al. Molecular Cloning and Characterization of the Human A3 Adenosine Receptor. *Proc. Natl. Acad. Sci.* **90**: 10365-10369 (1993) with modifications.

Accession Number:	GenBank L20463

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ADENOSINE, PURINERGIC₁, A₂ₐ (HUMAN RECOMBINANT) BINDING ASSAY

Reference Compounds	Ki (nM)
■ NECA	184.0
× CGS 21680	387.0
◆ 2-CADO	1265.0
◇ MECA	1582.0
▲ PIA	1636.0
□ AB-MECA	>10,000
● DIPY	>10,000

Assay Characteristics:

K_D (binding affinity):	75.0 nM
B_{max} (receptor number):	7 pmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in HEK 293 cells

Radioligand: [³H]CGS 21680 (30-60 Ci/mmol)
Final ligand concentration - [40.0 nM]

Non-specific Determinant: NECA - [50.0 μM]

Reference Compound: NECA

Positive Control: NECA

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 120 mM NaCl, 10 mM MgCl₂, 5 mM KCl and 2 mM CaCl₂ for 90 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned adenosine₂ₐ binding site.

Literature Reference: M. Jarvis, R. Schulz, A. Hutchison, U. Do, M. Sills, and M. Williams. [³H]CGS 21680: A Selective Adenosine 2 Receptor Agonist Directly Labels Adenosine 2 Receptors in Rat Brain. *Jrnl. Pharmacol. and Exper. Therap.* **251(3):** 888-893 (1989) with modifications.

Accession Number: GenBank X68486

ADENOSINE, PURINERGIC, P2y (HUMAN) BINDING ASSAY
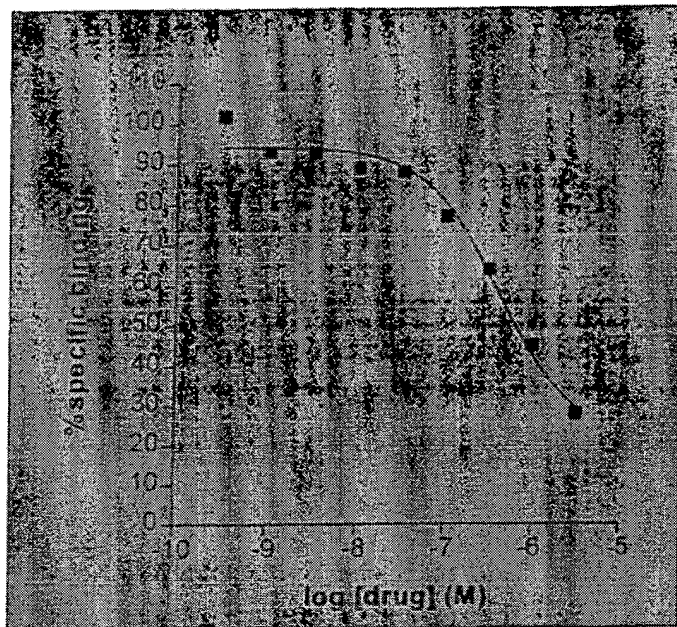
Reference Compounds	Ki (nM)
■ ADPβS	520.0

Assay Characteristics:

K_d (binding affinity):   0.3 nM
B_{max} (receptor number):   133 pmol/mg protein

Materials and Methods:

Receptor Source:	Human U937 cells
Radioligand:	[^{35}S] ADPβS (1100 - 1200 Ci/mmol)
	Final ligand concentration - [0.3 nM]
Non-specific Determinant:	ADPβS - [10 uM]
Reference Compound:	ADPβS
Positive Control:	ADPβS
Incubation Conditions:	Reactions are carried out in 10.0 mM HEPES (pH 7.4) at room temperature for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the P_{2Y} binding site.

Literature Reference:

Cooper, C.L., Morris, A.J., and Harden, T.K. Guanine Nucleotide-Sensitive Interaction of a Radiolabeled Agonist with a Phospholipase C-Linked P_{2Y}-Purinergic Receptor. *Jrnl. Biol. Chem.* **264**: 6202-6206 (1989) with modifications.

Levin, R.M., et al. High-Affinity, Divalent Ion-Specific Binding of [^{3}H]ATP to Homogenate Derived from Rabbit Urinary Bladder. *Mol. Pharmacol.* **23**: 1-7 (1983).

ADENOSINE, PURINERGIC, P$_{2Y}$
BINDING ASSAY
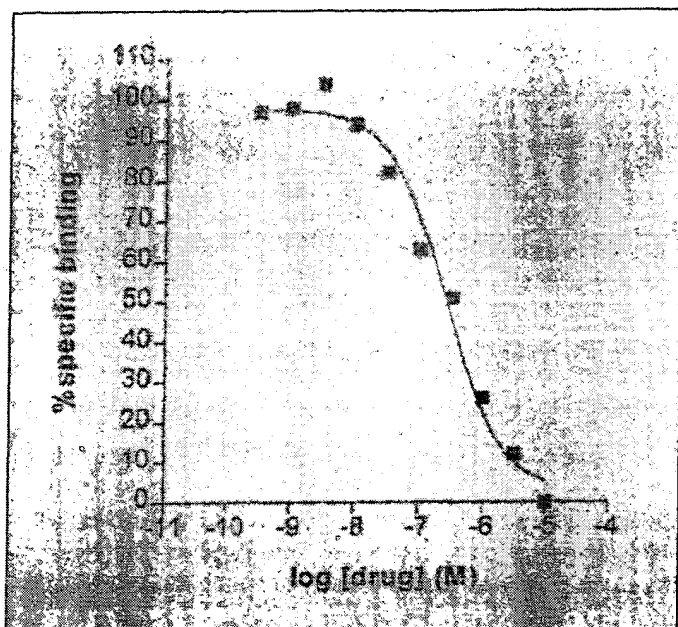
Reference Compounds	Ki(nM)
ADP	38.6
α, β-Methylene ATP	72.3
□ ADPβS	211.0
β, 0-Methylene ATP	256.0
2-Chloroadenosine	>10,000
Isobutylmethylxanthine	>10,000
8-Phenyltheophylline	>10,000

Assay Characteristics:

K$_D$ (binding affinity):	5.5 nM
B$_{max}$ (receptor number):	10.3 fmol/mg protein

Materials and Methods:

Receptor Source:	PC-12 cells
Radioligand:	[^{35}S]ADPβS (1100 - 1200 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specific Determinant:	ADPβS - [10 uM]
Reference Compound:	ADPβS
Positive Control:	ADPβS
Incubation Conditions:	Reactions are carried out in 10.0 mM HEPES (pH 7.4) at 30°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the P$_{2Y}$ binding site.

Literature Reference:	Cooper, C.L., Morris, A.J., and Harden, T.K. Guanine Nucleotide-Sensitive Interaction of a Radiolabeled Agonist with a Phospholipase C-Linked P$_{2Y}$-Purinergic Receptor. *Jrnl. Biol. Chem.* **264**: 6202-6206 (1989) with modifications.
	Levin, R.M., et al. High-Affinity, Divalent Ion-Specific Binding of [^{3}H]ATP to Homogenate Derived from Rabbit Urinary Bladder. *Mol. Pharmacol.* **23**: 1-7 (1983).

ADENOSINE TRANSPORT (HUMAN)
BINDING ASSAY
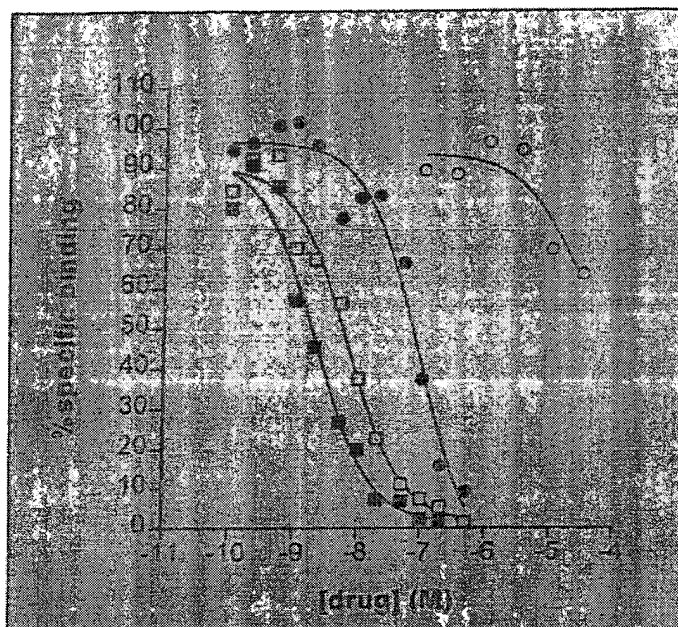
Reference Compound	Ki (nM)
□ NBTI	0.5
□ NBTG	1.7
○ DIPY	23.2
○ PIA	>300,000
Caffeine	>300,000
EHNA	>300,000
GBR12909	>300,000
2-CADO	>300,000
Theophylline	>300,000
Adenosine	>300,000
DMI	>300,000
NIPE	>300,000

Assay Characteristics:

K_d (binding affinity):	1.0 nM
B_{max} (receptor number):	2.4 pmol/mg protein

Materials and Methods:

Receptor Source:	Human U937 cells
Radioligand:	[^{3}H]Nitrobenzylthioinosine (15-25 Ci/mmol)
	Final ligand concentration - [3.0 nM]
Non-specific Determinant:	Nitrobenzylthioinosine (NBTI) - [1.0 µM]
Reference Compound:	Nitrobenzylthioinosine (NBTI)
Positive Control:	Nitrobenzylthioinosine (NBTI)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 22°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the adenosine uptake site.

Literature Reference:

Marangos, P. J., et al. Adenosine Uptake and [^{3}H]-NBTI Binding in Rat Brain. *Jrnl. Neurochem.* **39(1):** 184-191 (1982) with modifications.

ADENOSINE TRANSPORTER
BINDING ASSAY
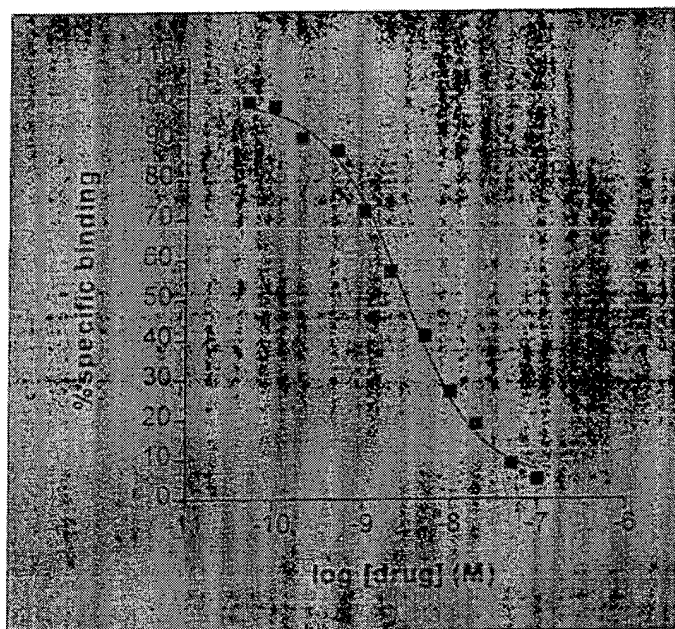
Reference Compounds	Ki (nM)
■ NBTI	2.7

Assay Characteristics:

K_d (binding affinity):   15.0 nM
B_{max} (receptor number):   135 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Nitrobenzylthioinosine (15-25 Ci/mmol)
	Final ligand concentration - [3.0 nM]
Non-specific Determinant:	Nitrobenzylthioinosine (NBTI) - [1.0 μM]
Reference Compound:	Nitrobenzylthioinosine (NBTI)
Positive Control:	Nitrobenzylthioinosine (NBTI)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 22°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the adenosine uptake site.

Literature Reference: Marangos, P. J., et al. Adenosine Uptake and [^{3}H]-NBTI Binding in Rat Brain. *Jrnl. Neurochem.* **39(1):** 184-191 (1982) with modifications.

ADENYLATE CYCLASE, FORSKOLIN
BINDING ASSAY
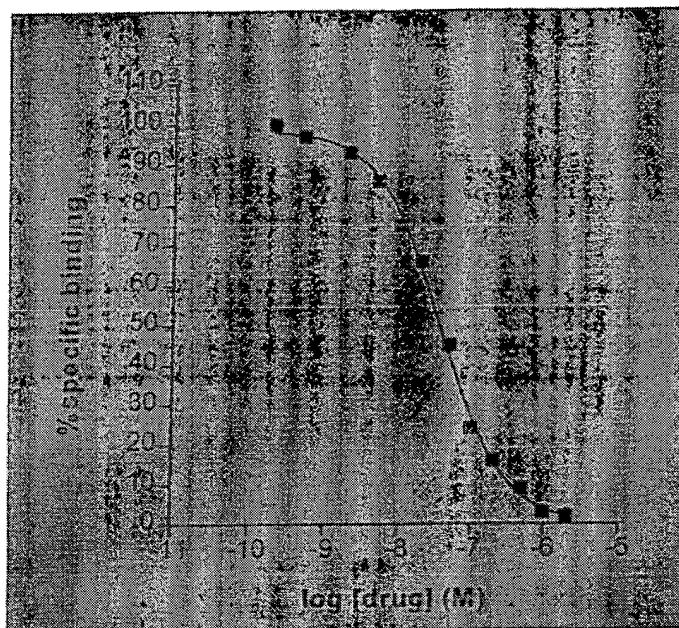
Reference Compounds	Ki (nM)
■ Forskolin	21.8
7-0-Hemisuccinyl-7-deacetylforskolin	51.3
7-β-deacetyl-7-β-butyrlforskolin	246.0
7-Deacetylforskolin	381.0
1,9-Dideoxyforskolin	>5,000

Assay Characteristics:

K_D (binding affinity):	22.8 nM
B_{max} (receptor number):	400 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[³H]Forskolin (20-40 Ci/mmol)
	Final ligand concentration - [10 nM]
Non-specific Determinant:	Forskolin - [5.0 uM]
Reference Compound:	Forskolin
Positive Control:	Forskolin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5.0 mM MgCl₂ at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the forskolin binding site.

Literature Reference:

Seamon, K.H., Vaillancourt, R., Edwards, M., and Daly, J.W. Binding of [³H]Forskolin to Rat Brain Membranes. *Proc. Nat'l Acad. of Sci.* **81**: 5081-5085 (1984) with modifications.

Seamon. K.B. and Daly, J.W. High-Affinity Binding of Forskolin to Rat Membranes. *Adv. Cyclic Nucleotide Prot. Phosphor. Res.* **19**: 125-135 (1985).

ADENOSINE TRANSPORT (HUMAN)
ASSAY
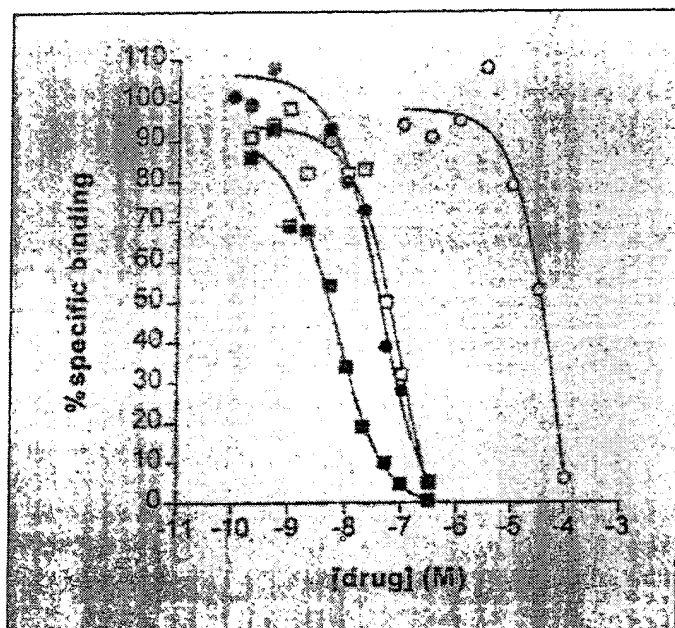
Reference Compound	Ki (nM)
NBTI	2.5
NBTG	20.5
Dipyridamole (DIPY)	25.0
Adenosine	15,000.0
Theophylline	22,500.0
R(-)-(2-Phenylisopropyl) adenosine (PIA)	32,000.0
2-Chloroadenosine (2-CADO)	42,500.0
EHNA	>100,000.0
GBR12909	>100,000.0
Caffeine	>100,000.0
Desipramine (DMI)	>100,000.0
Nipecotic	>1,000,000.0

Assay Characteristics:

K_t (transport affinity):	10.0 μM
V_{max} (transport rate):	83 pmol/min/mg protein

Materials and Methods:

Receptor Source:	U937 Cells
Substrate:	[^{3}H]Adenosine (0.01-0.1 Ci/mmol)
	Final substrate concentration - [10 μM]
Non-specific Determinant:	Nitrobenzylthioinosine (NBTI) - [1.0 μM]
Reference Compound:	Nitrobenzylthioinosine (NBTI)
Positive Control:	Nitrobenzylthioinosine (NBTI)
Incubation Conditions:	Reactions are carried out in KRH (pH 7.4) at 37°C for 30 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with adenosine uptake.

Literature References:

Gu, J.G., Nath, A., and Geiger, J.D. Characterization of Inhibitor - Sensitive and - Resistant Adenosine Transporters in Cultured Human Fetal Astrocytes. *Jrnl. Neurochem.* **(67)**, 972-977 (1996); with modifications.

Marangos, P. J., et al. Adenosine Uptake and [^{3}H]-NBTI Binding in Rat Brain. *Jrnl. Neurochem.* **39(1)**: 184-191 (1982) with modifications.

ADRENERGIC, ALPHA₁ₐ
BINDING ASSAY
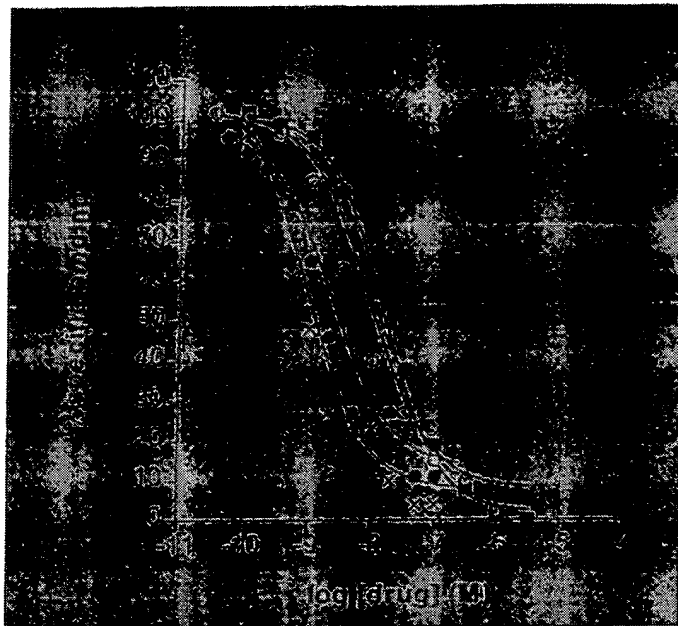
Reference Compounds	Ki (nM)
× Prazosin	1.5
● WB4101	3.0
○ 5-Methylurapidil	3.4
■ Phentolamine	8.3

Assay Characteristics:

K_D (binding affinity):   0.4 nM

B_{max} (receptor number):   191 fmol/mg protein

Materials and Methods:

Receptor Source: Rat cortical membranes (pretreated with chlorethyl clonidine-CEC)

Radioligand: [³H]-Prazosin (70-87 Ci/mmol)

Final ligand concentration - [0.4 nM]

Non-specific Determinant: Phentolamine - [10 μM]

Reference Compound: Phentolamine

Positive Control: Phentolamine

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 1 mM EDTA at 30 °C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha₁ₐ adrenergic binding site.

Literature Reference:

Gross, et al. 5-Methyl-Urapidil Discriminates Between Subtypes of the Alpha-1 Adrenoceptor. *Eur. Jrnl. Pharm.* **151**: 333-335 (1988).

Hanft,et al. Subclassification of Alpha-1 Adrenoceptor Recognition Sites by Urapidil Derivatives and other Selective Antagonists. *Brit. Jrnl. Pharm.* **97**: 691-700 (1989).

Minneman, et al. Comparison of Alpha-1 Adrenergic Receptor Subtypes Distinguished by Chlorethyl Clonidine. *Mol. Pharmacol.* **33**: 509-514.

ADRENERGIC, ALPHA₁, NON-SELECTIVE
BINDING ASSAY

Reference Compound	Ki (nM)
■ Prazosin	0.3
WB4101	2.6
♦ Phentolamine	5.4
Yohimbine	79.3

Assay Characteristics:

K_D (binding affinity):	0.2 nM
B_{max} (receptor number):	95 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[³H]Prazosin (70-87 Ci/mmol)
	Final ligand concentration - [0.3 nM]
Non-specific Determinant:	Prazosin - [1.0 μM]
Reference Compound:	Prazosin
Positive Control:	Prazosin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha₁ adrenergic binding site.

Literature Reference:

Timmermans, P., Ali, F.K., Kwa, H.Y., Schoop, A.M.C., Slothorst-Grisdijk, F.P. and van Zwieten, P.A. Identical Antagonist Selectivity of Central and Peripheral Alpha₁-Adrenoceptors. *Mol. Pharmacol.* **20**: 295-301 (1981) with modifications.

Reader, T.A., Briere, R., and Grondin, L. Alpha₁ and Alpha₂ Adrenoceptor Binding in Cerebral Cortex: Competition Studies with [³H]Prazosin and [³H]Idazosan. *Jrnl. Neural Transmission.* **68**: 79-95 (1987).

ADRENERGIC, ALPHA₂, NON-SELECTIVE
BINDING ASSAY
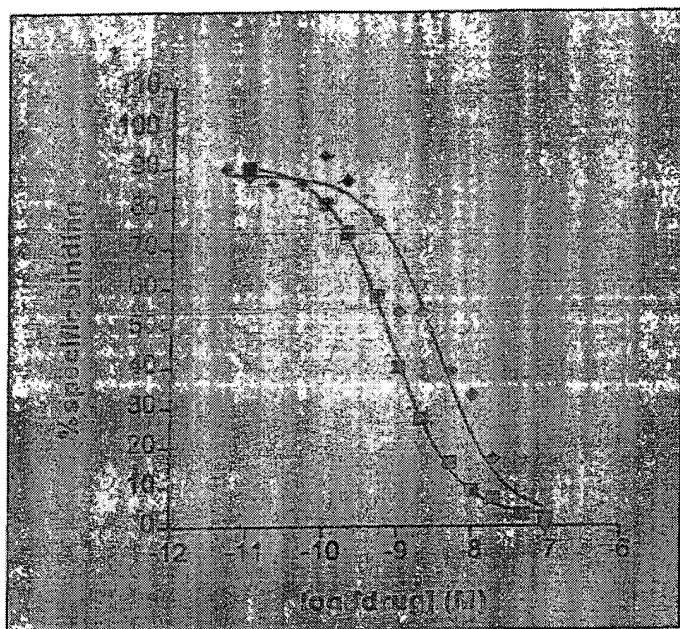
Reference Compounds	Ki (nM)
▣ RX 821002	0.5
RX 781094	3.0
◇ Phentolamine	3.2
Norepinephrine	42.0
Agmatine	563.0

Assay Characteristics:

K_D (binding affinity):	1.5 nM
B_{max} (receptor number):	60 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[³H]RX 821002 (40-60 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	RX 821002 - [0.1 μM]
Reference Compound:	RX 821002
Positive Control:	RX 821002
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 75 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha₂ adrenergic binding site.

Literature Reference:

O'Rourke, M.F., Blaxall, H.S., Iversen, L.J. and Bylund, D.B. Characterization of [³H]RX821002 to Alpha-2 Adrenergic Receptor Subtypes. *Jrnl. Pharmacol. Exp. Ther.* **268(3):** 1362 (1993) with modifications.

Reader, T.A., Briere, R., and Grondin, L. Alpha₁ and Alpha₂ Adrenoceptor Binding in Cerebral Cortex: Competition Studies with [³H]Prazosin and [³H]Idazoxan. *Jrnl. Neural Transmission* **68:** 79-95 (1987).

ADRENERGIC, ALPHA$_{1B}$
BINDING ASSAY
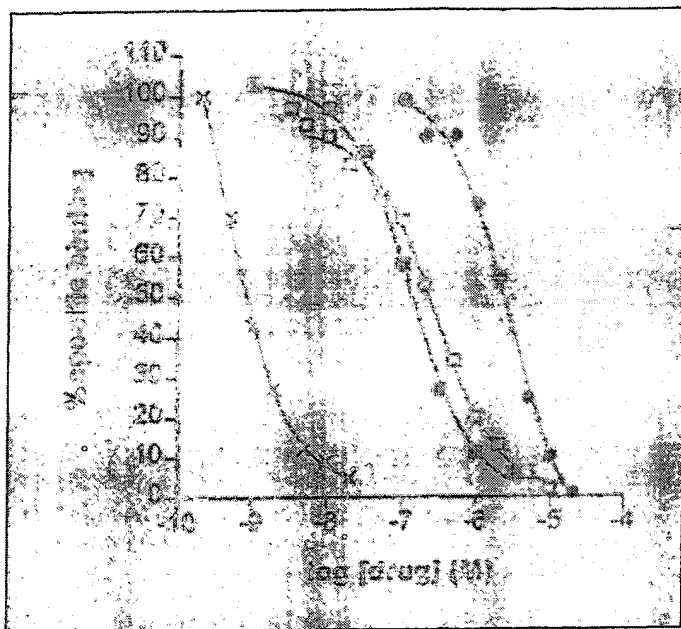
Reference Compounds		Ki (nM)
×	Prazosin	0.2
□	Phentolamine	47.5
□	5-Methylurapidil	90.0
○	Niguldipine	981.0

Assay Characteristics:

K_D (binding affinity):	0.2 nM
B_{max} (receptor number):	292 fmol/mg protein

Materials and Methods:

|	
Receptor Source:	Rat liver membranes
Radioligand:	[^{3}H]-Prazosin (70-87 Ci/mmol)
	Final ligand concentration - [0.4 nM]
Non-specific Determinant:	Phentolamine - [10 µM]
Reference Compound:	Phentolamine
Positive Control:	Phentolamine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 1 mM EDTA at 30 °C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha$_{1B}$ adrenergic binding site.

Literature Reference:

Gross, et al. 5-Methyl-Urapidil Discriminates Between Subtypes of the Alpha-1 Adrenoceptor. *Eur. Jrnl. Pharm.* **151**: 333-335 (1988).

Hanft, et al. Subclassification of Alpha-1 Adrenoceptor Recognition Sites by Urapidil Derivatives and other Selective Antagonists. *Brit. Jrnl. Pharm.* **97**: 691-700 (1989).

Minneman, et al. Comparison of Alpha-1 Adrenergic Receptor Subtypes Distinguished by Chlorethyl Clonidine. *Mol. Pharmacol.* **33**: 509-514.

ADRENERGIC, ALPHA$_{2A}$ (HUMAN RECOMBINANT) BINDING ASSAY

Reference Compounds	Ki (nM)
■ Yohimbine	7.0
Δ Oxymetazoline	11.0
▼ Prazosin	3529.0

Assay Characteristics:

K$_D$ (binding affinity):   0.5 nM
B$_{max}$ (receptor number):   23.5 fmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in SF9 cells
Radioligand: [^{3}H]MK-912 (60-80 Ci/mmol)
                            Final ligand concentration - [0.75 nM]
Non-specific Determinant: L(-)-norepinephrine - [100 uM]
Reference Compound: Oxymetazoline
Positive Control: Oxymetazoline
Incubation Conditions: Reactions are carried out in 75 mM TRIS-HCl (pH 7.5) containing 12.5 mM MgCl$_2$ and 2 mM EDTA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha$_{2A}$ adrenergic binding site.

Literature Reference:

Bylund, D.B., Prenger, C.R. and Murphy,T. J. Alpha-2A and Alpha-2B Adrenergic Receptor Subtypes. Antagonist Binding in Tissues and Cell Lines Containing only One Subtype. *Jrnl. Pharmac. & Exp. Ther.* **245(2):** 600-607 (1988) with modifications.

Totaro, J.A., Huff, J.R., Flagg, S.D., and Chen, R. [^{3}H]L-657,743 (MK-912): A New, High Affinity Selective Radioligand for Brain Alpha-2 Adrenoceptors. *Life Sciences.* **44:** 459-467 (1989) with modifiactions.

ADRENERGIC, ALPHA $_{2A}$
BINDING ASSAY

Reference Compounds	Ki (nM)
✕ Guanabenz acetate	2.2
■ Oxymetazoline	2.7
▲ Efaroxan	4.6
○ UK14304	10.3
◆ Prazosin	946.0

Assay Characteristics:

K_D (binding affinity):	0.5 nM
B_{max} (receptor number):	196 fmol/mg protein

Materials and Methods:

Receptor Source:	HT29 cells
Radioligand:	[^{3}H]MK-912 (60-80 Ci/mmol)
	Final ligand concentration - [0.75 nM]
Non-specific Determinant:	L(-)-Norepinephrine - [100 uM]
Reference Compound:	Oxymetazoline
Positive Control:	Rauwolscine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.5) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha$_{2A}$ adrenergic binding site.

Literature Reference:

Bylund, D.B., Prenger, C.R. and Murphy, T. J. Alpha-2A and Alpha-2B Adrenergic Receptor Subtypes. Antagonist Binding in Tissues and Cell Lines Containing only One Subtype. *Jrnl. Pharmac. & Exp. Ther.* **245(2):** 600-607 (1988) with modifications.

Totaro, J.A., Huff, J.R., Flagg, S.D., and Chen, R. [^{3}H]L-657,743 (MK-912): A New, High Affinity Selective Radioligand for Brain Alpha-2 Adrenoceptors. *Life Sciences.* **44:** 459-467 (1989).

ADRENERGIC, ALPHA₂C
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Raulwoscine	0.18
▲ Yohimbine	0.58
◆ Spiroxatrine	0.42
▼ Oxymetazoline	27.3
● Prazosin	72.6

Assay Characteristics:

K_D (binding affinity):	0.1 nM
B_{max} (receptor number):	20 fmol/mg protein

Materials and Methods:

|	
Receptor Source:	OK cells
Radioligand:	[^{3}H]MK-912 (60-80 Ci/mmol)
	Final ligand concentration - [0.75 nM]
Non-specific Determinant:	L(-)-norepinephrine - [100 μM]
Reference Compound:	Spiroxatrine
Positive Control:	Raulwoscine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.5) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha₂c adrenergic binding site.

Literature Reference:

Bylund, D.B., Prenger, C.R. and Murphy, T. J. Alpha-2A and Alpha-2B Adrenergic Receptor Subtypes. Antagonist Binding in Tissues and Cell Lines Containing only One Subtype. *Jrnl. Pharmac. & Exp. Ther.* **245(2):** 600-607 (1988) with modifications.

Totaro, J.A., Huff, J.R., Flagg. S.D., and Chen, R. [^{3}H]L-657,743 (MK-912): A New, High Affinity Selective Radioligand for Brain Alpha-2 Adrenoceptors. *Life Sciences.* **44:** 459-467 (1989) with modifications.

Bylund, D.B., et al. Characterization of [^{3}H]RX821002 Binding to Alpha-2 Adrenergic Receptor Subtypes. *J. Pharmacol. Exp. Therap.* **268:** 1362-1367 (1994) with modifications.

ADRENERGIC, ALPHA $_{2B}$
BINDING ASSAY
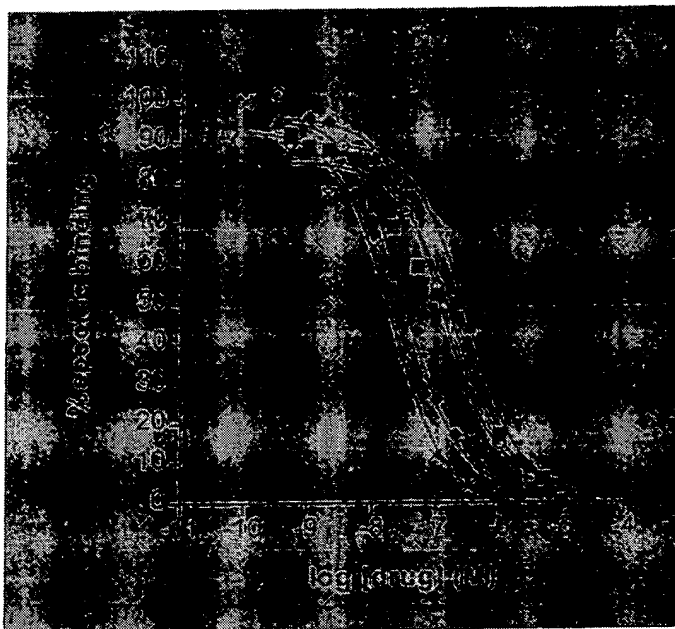
Reference Compounds		Ki (nM)
×	Guanabenz acetate	8.9
♦	Prazosin	14.1
○	UK14304	48.3
■	Oxymetazoline	80.0
▲	Efaroxan	136.3

Assay Characteristics:

K_D (binding affinity):	0.5 nM
B_{max} (receptor number):	126 fmol/mg protein

Materials and Methods:

Receptor Source:	NG108 cells
Radioligand:	[^{3}H]MK-912 (60-80 Ci/mmol)
	Final ligand concentration - [0.75 nM]
Non-specific Determinant:	L-(-)-Norepinephrine - [100 µM]
Reference Compound:	Oxymetazoline
Positive Control:	Rauwolscine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.5) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha$_{2B}$ adrenergic binding site.

Literature Reference:

Bylund, D.B., Prenger, C. R. and Murphy, T.J. Alpha-2A and Alpha-2B Adrenergic Receptor Subtypes. Antagonist Binding in Tissues and Cell Lines Containing only One Subtype. *Jrnl. Pharmacology. & Exp. Ther.* **245(2):** 600-607 (1988) with modifications.

Totaro, J.A., Huff, J.R., Flagg, S.D., and Chen, R. [^{3}H]L-657,743 (MK-912): A New, High Affinity Selective Radioligand for Brain Alpha-2 Adrenoceptors. *Life Sciences.* **44:** 459-467 (1989).

ADRENERGIC, BETA, NON-SELECTIVE
BINDING ASSAY
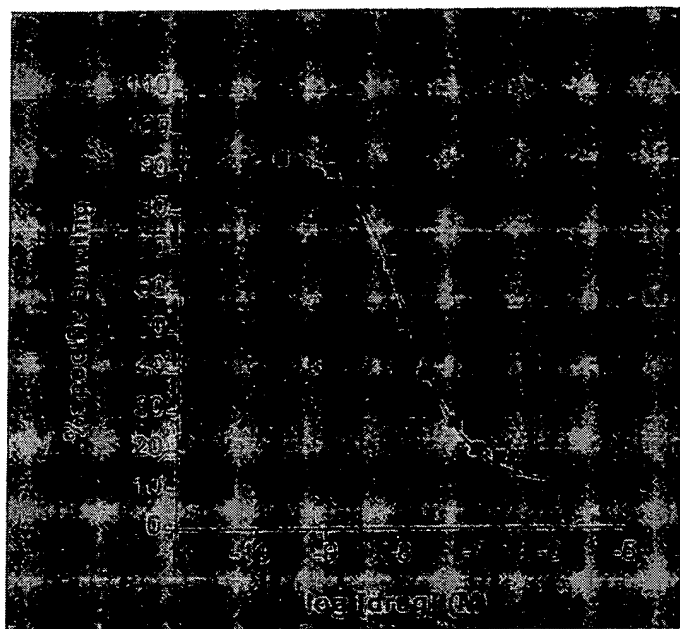
Reference Compounds	Ki (nM)
Pindolol	1.1
■ Alprenolol	7.7
Metoprolol	45.0
Isoproterenol	80.0
Albuterol	2,880.0

Assay Characteristics:

K_D (binding affinity): 1.7 nM

B_{max} (receptor number): 2.3 fmol/mg of tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [^{3}H]DHA (90-120 Ci/mmol)

Final ligand concentration - [2.0 nM]

Non-specific Determinant: Alprenolol - [10 u M]

Reference Compound: Alprenolol

Positive Control: Alprenolol

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 60 minutes for 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the beta adrenergic binding site.

Literature Reference:

M. Riva and I. Creese. Reevaluation of the Regulation of ß-Adrenergic Receptor Binding by Desipramine Treatment. *Mol. Pharmacol.* **36:** 211-218 (1989) with modifications.

Arango, V., Ernsberger, P., Reis, D. J., and Mann, J. J. Demonstration of High and Low Affinity ß-Adrenergic Receptors in Slide Mounted Sections of Rat and Human Brain. *Brain Res.* **516:** 113-121 (1990).

ADRENERGIC, ALPHA$_{2C}$ (HUMAN RECOMBINANT) BINDING ASSAY

Reference Compounds	Ki (nM)
× Yohimbine	3.8
△ Prazosin	87.3
■ Oxymetazoline	131.5

Assay Characteristics:

K_D (binding affinity):   0.09 nM

B_{max} (receptor number):   17.5 fmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in SF9 cells

Radioligand: [^{3}H]MK-912 (60-80 Ci/mmol)

Final ligand concentration - [0.75 nM]

Non-specific Determinant: L-(-)-Norepinephrine - [100 uM]

Reference Compound: Oxymetazoline

Positive Control: Oxymetazoline

Incubation Conditions: Reactions are carried out in 75 mM TRIS-HCl (pH 7.5) containing 12.5 mM MgCl$_2$ and 2 mM EDTA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the alpha$_{2C}$ adrenergic binding site.

Literature Reference: Bylund, D.B., Prenger, C.R.and Murphy,T. J.Alpha-2Aand Alpha-2B Adrenergic Receptor Subtypes. Antagonist Binding in Tissues and Cell Lines Containing only One Subtype. *Jrnl. Pharmac. & Exp. Ther.* **245(2):** 600-607 (1988) with modifications.

Totaro, J.A., Huff, J.R., Flagg, S.D., and Chen, R. [^{3}H]L-657,743 (MK-912): A New, High Affinity Selective Radioligand for Brain Alpha-2 Adrenoceptors. *Life Sciences.* **44:** 459-467 (1989) with modifications.

ADRENERGIC, BETA, (HUMAN RECOMBINANT) BINDING ASSAY

Reference Compounds	Ki (nM)
□ ICI 89,406	0.7
□ Alprenolol	2.6
× ICI 118,551	280.1
◇ Albuterol	2816.0

Assay Characteristics:

K_D (binding affinity):	0.05 nM
B_{max} (receptor number):	85 fmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expessed in CHO-REX16 cells
Radioligand:	[^{125}I] (-) Iodocyanopindolol (2000 Ci/mmol)
	Final ligand concentration - [0.05 nM]
Non-specific Determinant:	Alprenolol HCl - [1.0 μM]
Reference Compound:	Alprenolol HCl
Positive Control:	Alprenolol HCl
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4), containing, 12 mM $MgCl_2$, and 2 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned beta$_1$ adrenergic site.

Literature Reference:

R.N. Kalaria, A.C. Andorn, M. Tabaton, P.J. Whitehouse, S.I. Harik, J.R. Unnerstall. Adrenergic Receptors in Aging and Alzheimer's Disease: Increased β_2-Receptors in Prefrontal Cortex and Hippocampus. *Journal of Neurochemistry.* **53**: 1772-1781 (1989) with modifications.

Minneman, K.P., Hegstrand, L.R., and Molinoff, P.B. Simultaneous Determination of Beta$_1$ and Beta$_2$ Adrenergic Receptors in Tissues Containing Both Receptor Subtypes. *Mol. Pharmacol.* **16**: 34-46 (1979).

ADRENERGIC, BETA₁
BINDING ASSAY
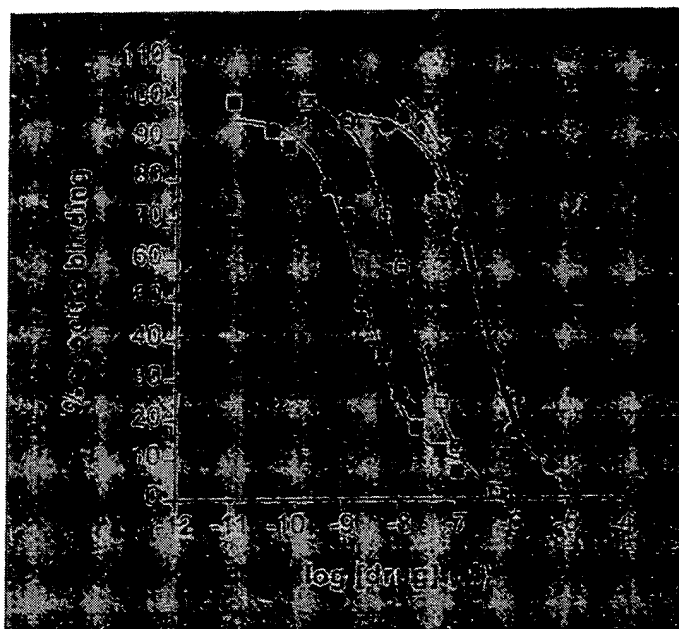
Reference Compounds	Ki (nM)
■ ICI-89,406	1.1
□ Alprenolol	14.8
● (-) Isoproterenol (+) bitartrate	213.0
× ICI-118,551	460.0

Assay Characteristics:

K_D (binding affinity): 5.4 nM
B_{max} (receptor number): 7.7 pmol/mg protein

Materials and Methods:

Receptor Source: Rat cortical membranes
Radioligand: [^{125}I] (-) Iodopindolol (2200 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific Determinant: Alprenolol HCl - [10 μM]
Reference Compound: Alprenolol HCl
Positive Control: Alprenolol HCl
Blocker: ICI-118,551 (Beta₂ blocker) [120 nM]
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.5), containing 150 mM NaCl, 2.5 mM MgCl₂, and 0.5 mM ascorbate for 60 minutes at 37°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the beta₁ adrenergic site.

Literature Reference: R.N. Kalaria, A.C. Andorn, M. Tabaton, P.J. Whitehouse, S.I. Harik, J.R. Unnerstall. Adrenergic Receptors in Aging and Alzheimer's Disease: Increased β₂-Receptors in Prefrontal Cortex and Hippocampus. *Journal of Neurochemistry.* **53**: 1772-1781 (1989) with modifications.

Minneman, K.P., Hegstrand, L.R., and Molinoff, P.B. Simultaneous Determination of Beta₁ and Beta₂ Adrenergic Receptors in Tissues Containing Both Receptor Subtypes. *Mol. Pharmacol.* **16**: 34-46 (1979).

ADRENERGIC, BETA$_2$ (HUMAN RECOMBINANT)
BINDING ASSAY
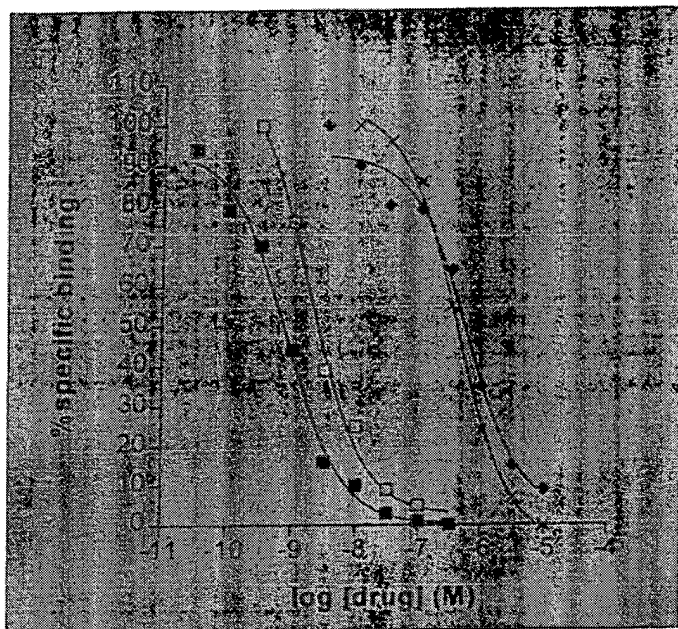
Reference Compounds	Ki (nM)
■ Alprenolol	0.36
□ ICI 118,551	0.75
× ICI 89,406	175.8
◆ Albuterol	254.0

Assay Characteristics:

K$_D$ (binding affinity):   0.04 nM
B$_{max}$ (receptor number):   150 fmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO-WT21 cells
Radioligand: [^{125}I] (-) Iodocyanopindolol (2000 Ci/mmol)
                          Final ligand concentration - [0.05 nM]
Non-specific Determinant: Alprenolol HCl - [1.0 µM]
Reference Compound: Alprenolol HCl
Positive Control: Alprenolol HCl
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4), containing, 12 mM MgCl$_2$, and 2 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned beta$_1$ adrenergic site.
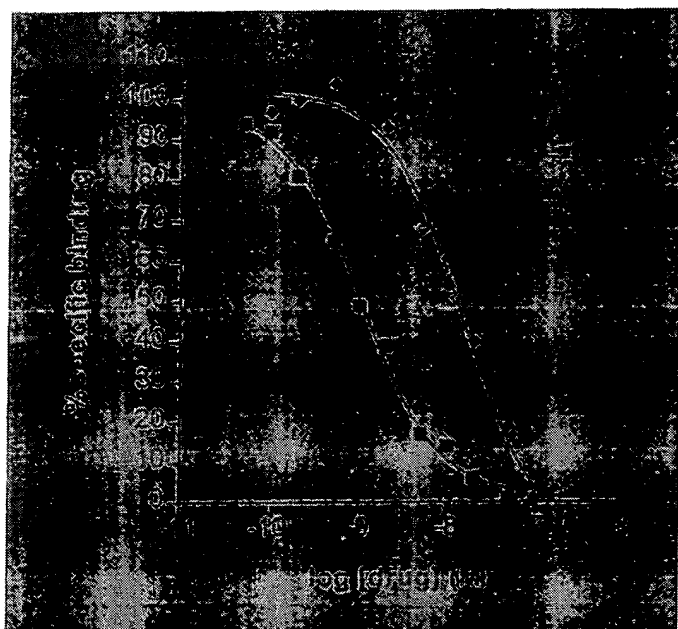
Literature Reference:

R.N. Kalaria, A.C. Andorn, M. Tabaton, P.J. Whitehouse, S.I. Harik, J.R. Unnerstall. Adrenergic Receptors in Aging and Alzheimer's Disease: Increased β$_2$-Receptors in Prefrontal Cortex and Hippocampus. *Journal of Neurochemistry.* **53**: 1772-1781 (1989) with modifications.

Minneman, K.P., Hegstrand, L.R., and Molinoff, P.B. Simultaneous Determination of Beta$_1$ and Beta$_2$ Adrenergic Receptors in Tissues Containing Both Receptor Subtypes. *Mol. Pharmacol.* **16**: 34-46 (1979).

ADRENERGIC, BETA$_2$
BINDING ASSAY
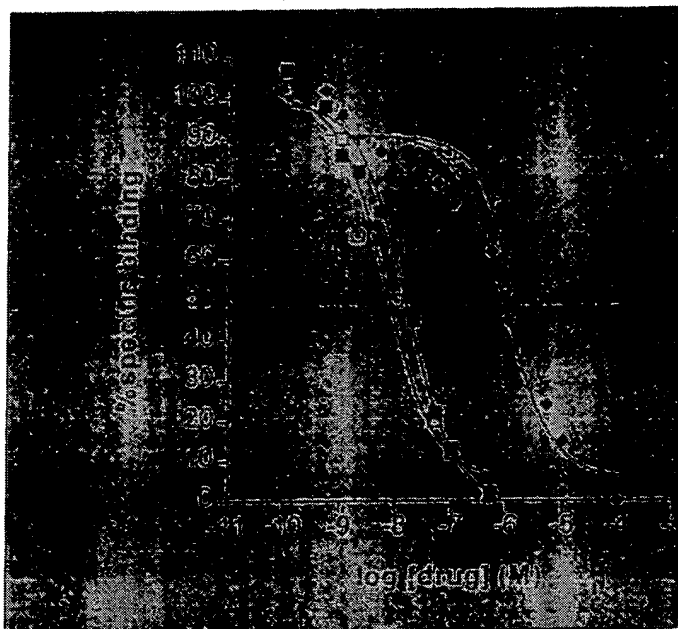
Reference Compounds	Ki (nM)
■ ICI-118,551	9.0
□ Alprenolol	10.7
◆ (-) Isoproterenol (+) bitartrate	633.0
+ ICI-89,406	3000.0

Assay Characteristics:

K_D (binding affinity):	4.4 nM
B_{max} (receptor number):	5.9 pmol/mg protein

Materials and Methods:

Receptor Source: Rat cortical membranes
Radioligand: [^{125}I] (-) Iodopindolol (2200 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific Determinant: Alprenolol HCl - [10 uM]
Reference Compound: Alprenolol HCl
Positive Control: Alprenolol HCl
Blocker: ICI-89,406 (Beta$_1$ blocker) [100 nM]
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.5), containing 150 mM NaCl, 2.5 mM MgCl$_2$, and 0.5 mM ascorbate for 60 minutes at 37°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the beta$_2$ adrenergic site.

Literature Reference: R.N. Kalaria, A.C. Andorn, M. Tabaton, P.J. Whitehouse, S.I. Harik, J.R. Unnerstall. Adrenergic Receptors in Aging and Alzheimer's Disease: Increased ß$_2$-Receptors in Prefrontal Cortex and Hippocampus. *Jrnl. Neurochem.* **53**: 1772-1781 (1989) wit modifications.

Minneman, K. P., Hegstrand, L.R., and Molinoff, P. B. Simultaneous Determination of Beta$_1$ and Beta$_2$ Adrenergic Receptors in Tissues Containing Both Receptor Subtypes. *Mol. Pharmacol.* **16**: 34-46 (1979).

ANGIOTENSIN II, TYPE 1, AT, (HUMAN)
BINDING ASSAY
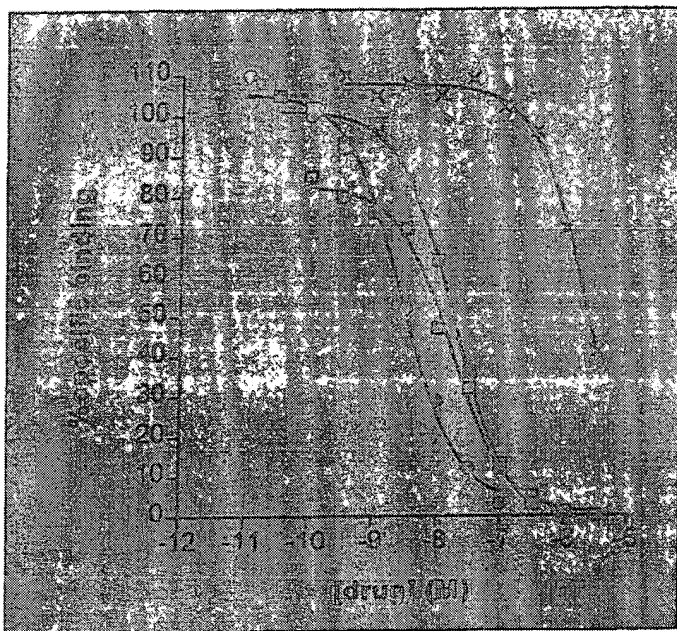
Reference Compound		Ki (nM)
o	DuP753	1.2
◻	Angiotensin I	7.8
◘	Angiotensin II	7.9
×	Angiotensin III	1,065

Assay Characteristics:

K_D (binding affinity): 0.08 nM
B_{max} (receptor number): 0.2 pmol/mg protein

Materials and Methods:

Receptor Source: Human KANTS cell membranes
Radioligand: Sar^1, $[^{125}I\text{-}Try^4]$, Ile^8 Angiotensin II (2200 Ci/mmol)
Final ligand concentration - [0.08 nM]
Non-specific Determinant: Angiotensin II - [1.0 μM]
Reference Compound: Angiotensin II
Positive Control: Angiotensin II
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 100 mM NaCl, 1 mM $MgCl_2$, 0.1 mM bacitracin and 0.1% BSA for 60 minutes at room temperature. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the Angiotensin - AT_1 binding site.
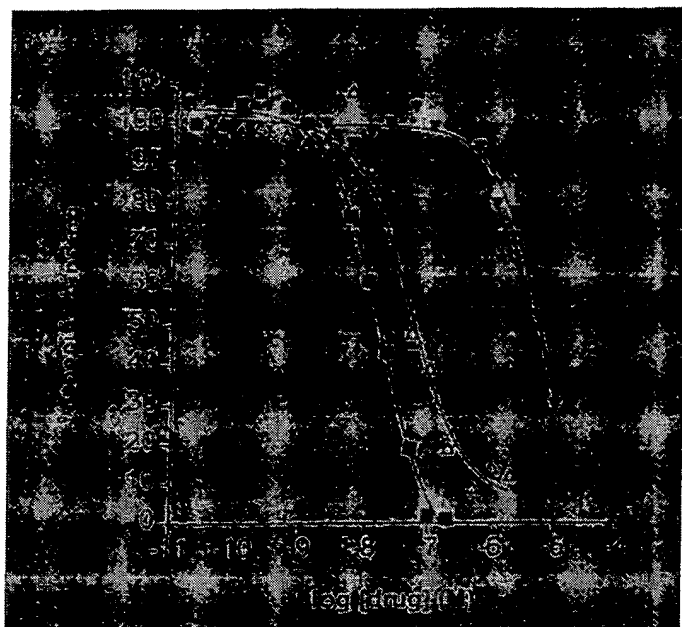
Literature Reference:

J.P. Bennett and S.H. Snyder. Angiotensin II Binding to Mammalian Brain Membranes. *Jrnl. Biol. Chem.* **251**: 7423-7430 (1976) with modifications.

Wiest, S.A., Rampersaud, A., Zimmerman, K., and Steinberg, M.I. Characterization of Distinct Angiotensin II Binding Sites in Rat Adrenal Gland and Bovine Cerebellum using Selective Nonpeptide Antagonists. *Jrnl. Cardio. Pharmacol.* **17(2)**: 177-184 (1991).

ANGIOTENSIN II, TYPE 1, PERIPHERAL (AT₁)
BINDING ASSAY
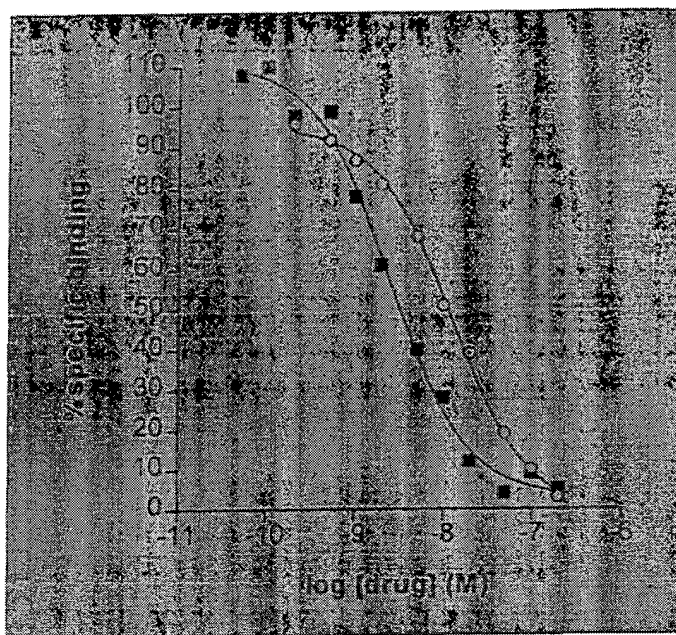
Reference Compounds	Ki (nM)
▪ Angiotensin II (human)	8.2
○ DuP753	9.3

Assay Characteristics:

K_d (binding affinity):	0.3 nM
B_{max} (receptor number):	112 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat liver membranes

Radioligand: [^{125}I] Tyr^4, Sar^1, ILe^8-Angiotensin II (2200 Ci/mmol)
Final ligand concentration - [20 pM]

Non-specific Determinant: Angiotensin II, human - [1.0 μM]

Reference Compound: Angiotensin II, human

Positive Control: DuP753

Incubation Conditions: Reactions are carried out in 50 mM TRIS HCl (pH 7.2) containing 100 mM NaCl, 1 mM $MgCl_2$, 0.1mM Bacitracin and 0.1% BSA for 3 hrs. at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the angiotensin II, type 1 (peripheral) binding site.

Literature Reference: J.P. Bennett and S.H. Snyder. Angiotensin II Binding to Mammalian Brain Membranes. *Jrnl. Biol. Chem.* **251**: 7423-7430 (1976) with modifications.

Wiest, S.A., Rampersaud, A., Zimmerman, K., and Steinberg, M.I. Characterization of Distinct Angiotensin II Binding Sites in Rat Adrenal Gland and Bovine Cerebellum using Selective Nonpeptide Antagonists. *Jrnl. Cardio. Pharmacol.* **17(2)**: 177-184 (1991).

ATRIAL NATRIURETIC PEPTIDE, ANP$_A$
BINDING ASSAY
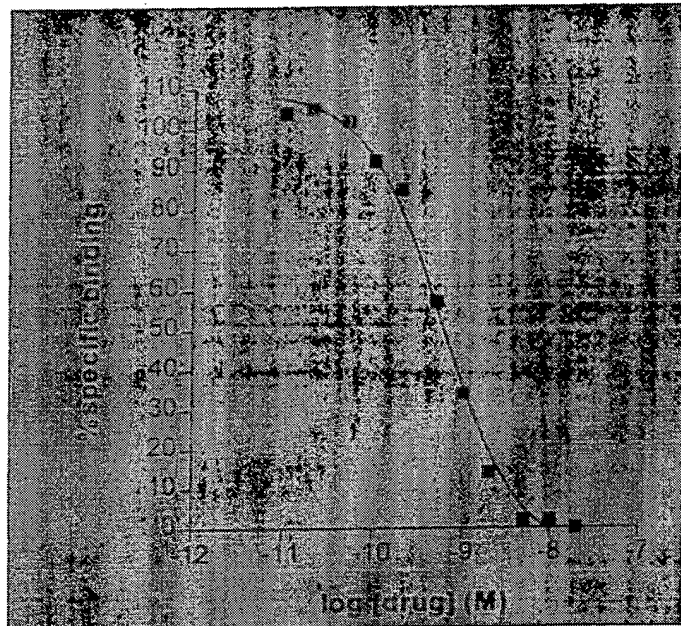
Reference Compounds	Ki (nM)
■ ANP (rat)	0.3
ANP $_{103-123}$	>1,000,000

Assay Characteristics:

K$_d$ (binding affinity):   0.06 nM
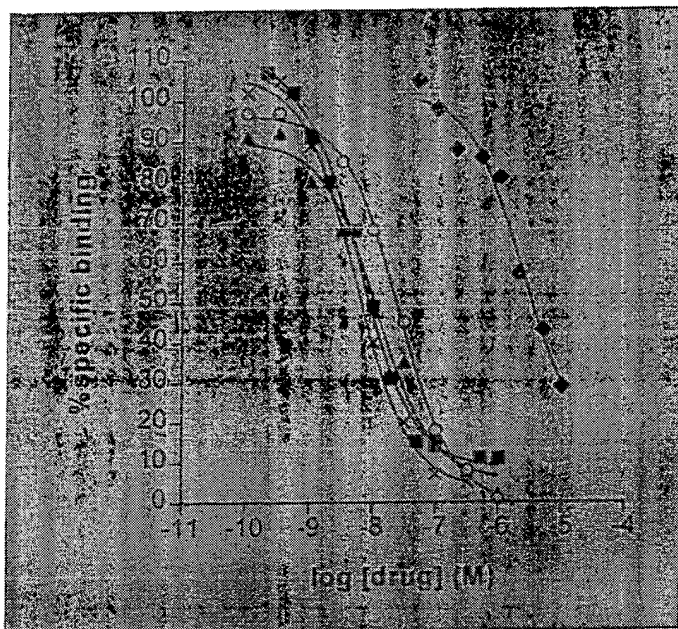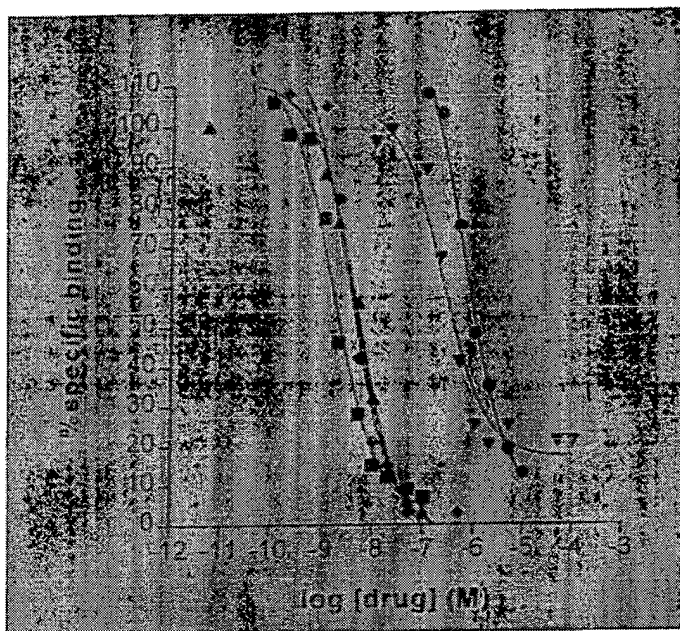B$_{max}$ (receptor number):   95 fmol/mg protein

Materials and Methods:

Receptor Source: Guinea pig cerebellar membranes

Radioligand: [^{125}I]Atrial natriuretic peptide (2200 Ci/mmol)
Final ligand concentration - [0.05 nM]

Non-specific Determinant: Atrial natriuretic peptide (rat) - [0.1 μM]

Reference Compound: Atrial natriuretic peptide (rat)

Positive Control: Atrial natriuretic peptide (rat)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 150 mM NaCl, 5 mM MnCl$_2$ and 0.5% BSA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the ANF$_A$ binding site.

Literature Reference:

Schiffrin, E.L.,et al. Vascular and Adrenal Receptors for Atrial Natriuretic Factor in the Rat. *Circulation Research.* **56:** 801-807 (1985) with modifications.

Fethiere, J., Meloche, S., et al. Distinct Properties of ANF Receptor Subpopulations in Epithelial & Fibroblast Cell Lines. *Mol. Pharmacol.* **35:** 584-592 (1989).

ANGIOTENSIN II, TYPE 2, CENTRAL (AT$_2$)
BINDING ASSAY
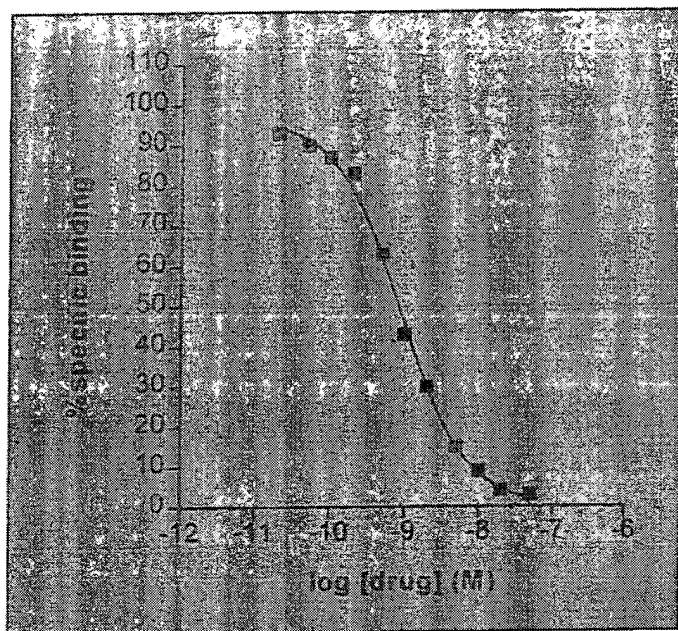
Reference Compounds	Ki (nM)
□ Angiotensin II (human)	0.4

Assay Characteristics:

K$_D$ (binding affinity):	0.4 nM
B$_{max}$ (receptor number):	2.11 fmol/mg tissue (wet weight)

Materials and Methods:

|	
Receptor Source:	Bovine cerebellar membranes
Radioligand:	[^{125}I]-Tyr^4-Angiotensin II (2200 Ci/mmol)
	Final ligand concentration - [0.1 nM]
Non-specifc Determinant:	Angiotensin II (Human) - [0.05 uM]
Reference Compound:	Angiotensin II (Human)
Positive Control:	Angiotensin II (Human)
Incubation Conditions:	Reactions are carried out in phosphate buffer (pH 7.0) containing NaCl, Na$_2$EDTA and BSA for 60 minutes at 37°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the angiotensin II, type 2 (central) binding site.

Literature Reference:

Bennett, J. P. and Snyder, S. H. Angiotensin II Binding to Mammalian Brain Membranes. *Jrnl. Biol. Chem.* **251**: 7423-7430 (1976) with modifications.

Wiest, S.A., Rampersaud, A., Zimmerman, K., and Steinberg, M.I. Characterization of Distinct Angiotensin II Binding Sites in Rat Adrenal Gland and Bovine Cerebellum using Selective Nonpeptide Antagonists. *Jrnl. Cardio. Pharmacol.* **17(2)**: 177-184 (1991).

BENZODIAZEPINE (PERIPHERAL, HUMAN)
BINDING ASSAY
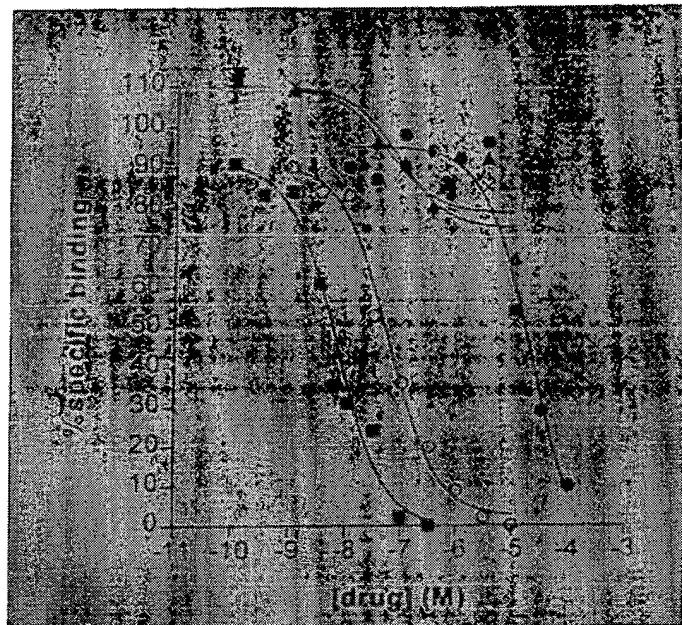
Reference Compound	Ki (M)
■ PK 11195	4.1
○ Ro 54864	34.5
● Clonazepam	12,300
× Flunitrezapam	>10,000
▲ Ro 151788	>10,000

Assay Characteristics:

K_D (binding affinity):	1.8 nM
B_{max} (receptor number):	12 pmol/mg protein

Materials and Methods:

Receptor Source:	Human colonic cell membranes
Radioligand:	[^{3}H]PK11195 (60-90 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	PK11195 - [1.0 uM]
Reference Compound:	PK11195
Positive Control:	PK11195
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) at RT for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the benzodiazepine (peripheral) binding site.

Literature Reference:

Skowronski, R., et al. Photoaffinity Labeling of Peripheral Type Benzodiazepine Receptors in Rat Kidney Mitochondria with [^{3}H]PK14105. *Eur. Jrnl. Pharmacology.* **148**: 187-193 (1988) with modifications.

Raghavendra Roa, V.L., Audet, R., Therrien, G., et al. Tissue Specific Alterations of Binding Sites for Peripheral Type Benzodiazepine Receptor Ligand [^{3}H]PK11195 in Rats Following Potacaval Anastomosis. *Digestive Diseases & Sciences.* **39(5)**: 1055-1063 (1994).

BENZODIAZEPINE (PERIPHERAL)
BINDING ASSAY
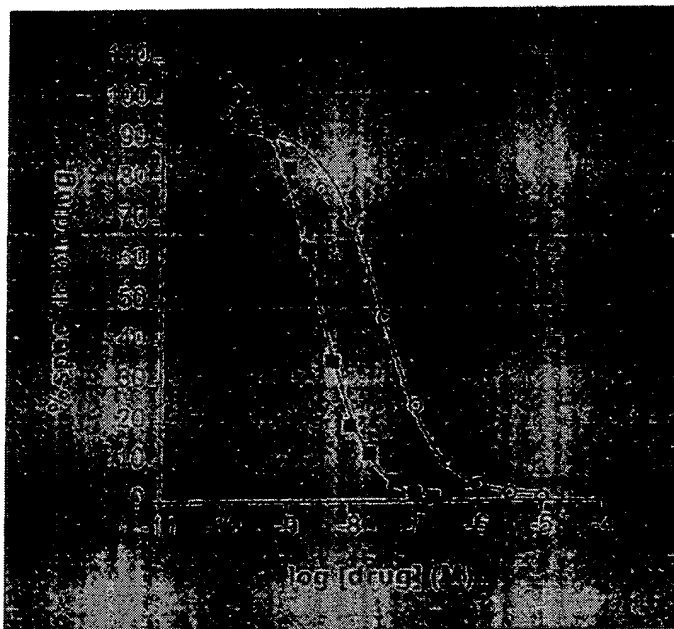
Reference Compounds	Ki (nM)
■ PK 11195	2.4
○ Diazepam	20.2
Ro 54864	34.0

Assay Characteristics:

K_D (binding affinity): 9.8 nM
B_{max} (receptor number): 23.9 fmol/mg tissue (wet weight)
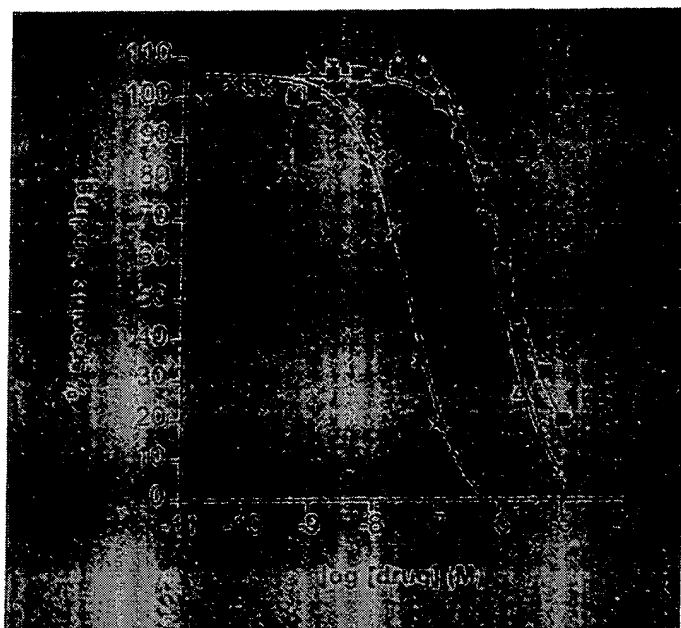
Materials and Methods:

Receptor Source: Rat kidney membranes
Radioligand: [^{3}H]PK11195 (60-90 Ci/mmol)
  Final ligand concentration - [1.0 nM]
Non-specific Determinant: PK11195 - [0.2 uM]
Reference Compound: PK11195
Positive Control: PK11195
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the benzodiazepine (peripheral) binding site.

Literature Reference: Skowronski, R., et al. Photoaffinity Labeling of Peripheral Type Benzodiazepine Receptors in Rat Kidney Mitochondria with [^{3}H]PK14105. *Eur. Jrnl. Pharmacology.* **148**: 187-193 (1988) with modifications.

Raghavendra Roa, V.L., Audet, R., Therrien, G., et al. Tissue Specific Alterations of Binding Sites for Peripheral Type Benzodiazepine Receptor Ligand [^{3}H]PK11195 in Rats Following Potacaval Anastomosis. *Digestive Diseases & Sciences.* **39(5)**: 1055-1063 (1994).

BRADYKININ, BK$_2$ (HUMAN RECOMBINANT) BINDING ASSAY
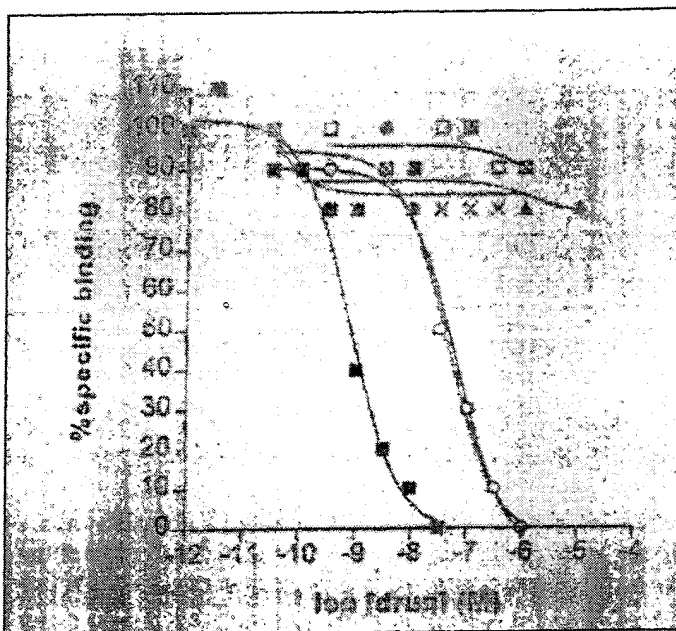
Reference Compounds	Ki (nM)
□ Bradykinin TFA salt	0.4
○ D-Arg^0,Hyp^3,Phe^7 BDKN	22.8
○ D-Arg^0,Hyp^3,Phe^7,Thi^{5,8} BDKN	31.8
× desArg^9,Leu^8 BDKN	>1,000
□ BDKN 1-5	>1,000
△ Substance P	>1,000

Assay Characteristics:

K$_D$ (binding affinity):	0.4 nM
B$_{max}$ (receptor number):	3 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO-K1 cells
Radioligand:	[^{3}H]Bradykinin (111.0 Ci/mmol) Final ligand concentraion - [0.4 nM]
Non-specific Determinant:	Bradykinin TFA salt - [0.1 µM]
Reference Compound:	Bradykinin TFA salt
Positive Control:	Bradykinin TFA salt
Incubation Conditions:	Reactions are carried out in 25 mM TES (pH 6.8)/ 1 mM 1,10 phenanthroline buffer containing 0.3% BSA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the B$_2$ binding site.
Literature Reference:	Manning, D., Vavrek, R., Stewart, J. and Snyder, S. H. Two Bradykinin Binding Sites with Picomolar Affinities. *Jrnl. Pharmacol. Exp. Therap.* **237**: 504-512 (1986) with modifications.

BRADYKININ, BK$_2$
BINDING ASSAY
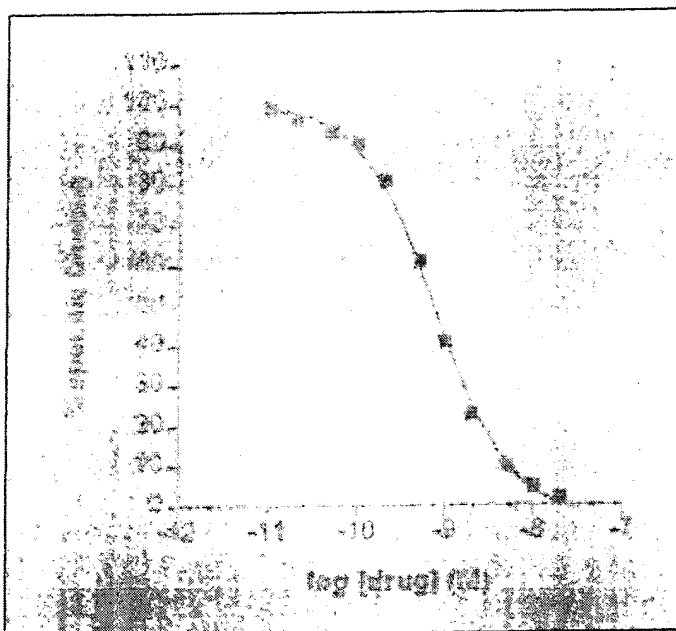
Reference Compounds	Ki (nM)
[D-Arg^9]-Bradykinin	0.1
□ Bradykinin TFA salt	0.2
[Lys^0]-BDKN (Kalladin)	0.4
[D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7]-BDKN	10.0
[D-Arg^0, Hyp^3, D-Phe^7]-BDKN	15.0
Substance P	>10,000
Angiotensin II	>10,000
[Arg^8]-Vasopressin (AVP)	>10,000
CCK$_8$	>10,000

Assay Characteristics:

K$_D$ (binding affinity):	0.4 nM
B$_{max}$ (receptor number):	12.3 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Guinea pig ileum membranes
Radioligand: [^{3}H]Bradykinin (111.0 Ci/mmol)
Final ligand concentraion - [0.2 nM]

Non-specific Determinant: Bradykinin TFA salt - [1.0 µM]
Reference Compound: Bradykinin TFA salt
Positive Control: Bradykinin TFA salt
Incubation Conditions: Reactions are carried out in 25 mM TES (pH 6.8)/ 1 mM, 1, 10 phenanthroline buffer containing 0.1 mM bacitracin, 0.1% BSA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the bradykinin binding site.

Literature Reference: Manning, D., Vavrek, R., Stewart, J. and Snyder, S. H. Two Bradykinin Binding Sites with Picomolar Affinities. *Jrnl. Pharmacol. Exp. Therap.* **237**: 504-512 (1986) with modifications.

Ransom, R.W., Young, G.S., Schneck, K., and Goodman, C.B. Characterization of Solubilized B2 Receptors from Smooth Muscle & Mucosa of Guinea Pig Ileum. *Biochem. Pharmacol.* **43(8):** 1823-1827 (1992).

CALCITONIN GENE RELATED PEPTIDE, CGRP (PERIPHERAL)
BINDING ASSAY
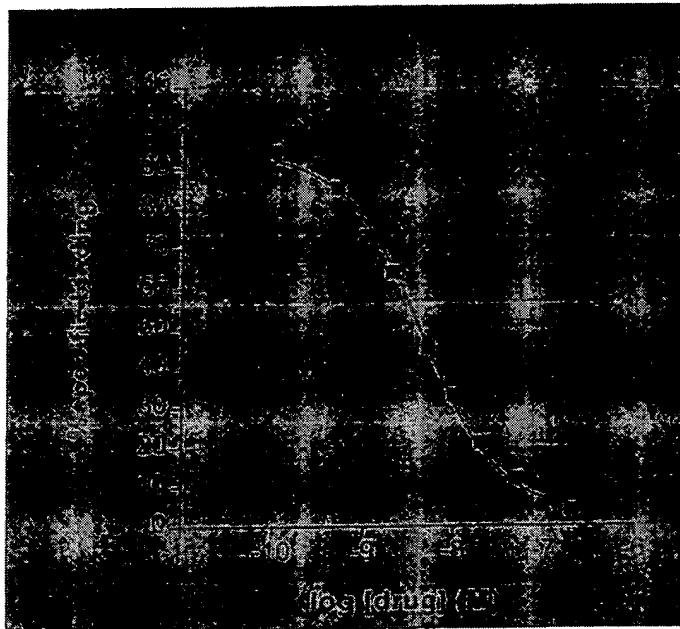
Reference Compounds............Ki (nM)

■ αCGRP, human (8-37)   3.3

Assay Characteristics:

K_0 (binding affinity):   6.4 nM
B_{max} (receptor number):   22.5 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat spleen membranes
Radioligand:	[^{125}I]-hCGRP (2000 Ci/mmol)
	Final ligand concentration - [0.1 nM]
Non-specific Determinant:	αCGRP, human (8-37) - [1.0 mM]
Reference Compound:	αCGRP, human (8-37)
Positive Control:	αCGRP, human (8-37)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) for 2 hours at 0-4°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters and radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the CGRP peripheral binding site.

Literature Reference:

T. Dennis, A. Fournier, S. St. Pierre, and R. Quirion. Structure-Activity Profile of Calcitonin Gene-Related Peptide in Peripheral and Brain Tissues. Evidence for Receptor Multiplicity. *Jrnl. Pharmacol. Exp. Therap.* **251(2)**: 718-725 (1989).

Wimalawansa, S.J. Emson, P.C., MacIntyre, I. Regional Distribution of CGRP and its Specific Binding Sites in Rats with Particular Reference to the Nervous System. *Neuroendocrin.* **46**: 131-136 (1987).

CALCITONIN GENE RELATED PEPTIDE, CGRP (CENTRAL)
BINDING ASSAY
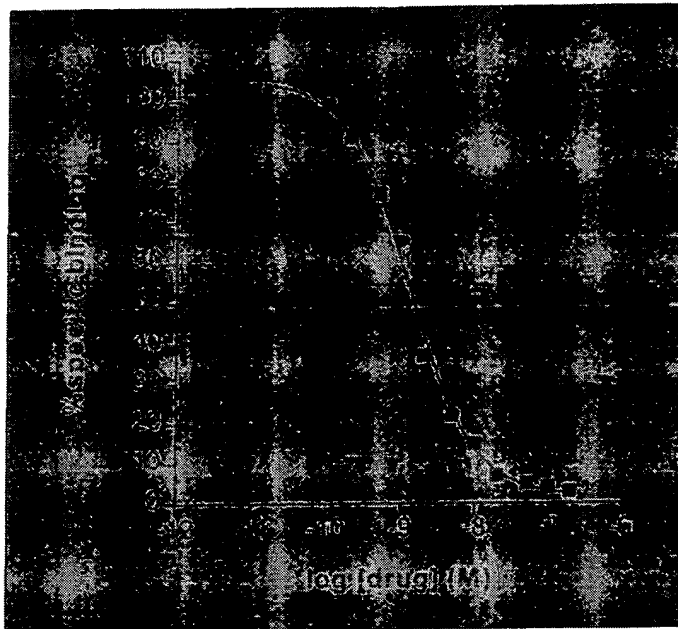
Reference Compounds	Ki (nM)
■ αCGRP, human (8-37)	1.6

Assay Characteristics:

K_D (binding affinity):   1.7 nM

B_{max} (receptor number):   7.5 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat forebrain membranes

Radioligand: [^{125}I]-hCGRP (2000 Ci/mmol)

Final ligand concentration - [0.1 nM]

Non-specific Determinant: αCGRP, human (8-37) - [1.0 µM]

Reference Compound: αCGRP, human (8-37)

Positive Control: αCGRP, human (8-37)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4 for 2 hours at 0-4°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters and radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the CGRP central binding site.

Literature Reference: T. Dennis, A. Fournier, S. St. Pierre, and R. Quirion. Structure-Activity Profile of Calcitonin Gene-Related Peptide in Peripheral and Brain Tissues. Evidence for Receptor Multiplicity. *Jrnl. Pharmacol. Exp. Therap.* **251(2):** 718-725 (1989).

Wimalawansa, S.J. Emson, P.C., MacIntyre, I. Regional Distribution of CGRP and its Specific Binding Sites in Rats with Particular Reference to the Nervous System. *Neuroendocrin.* **46:** 131-136 (1987).

CALCIUM CHANNEL, TYPE L (DIHYDROPYRIDINE SITE)
BINDING ASSAY
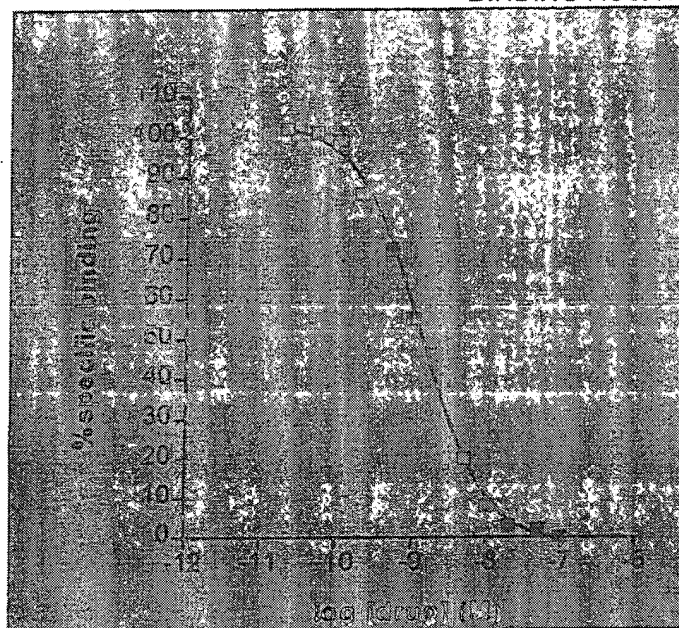
Reference Compounds	Ki (nM)
▣ Nifedipine	0.8
Saxitoxin	13.8
ω-Conotoxin	>10,000
Apamin	>10,000
TBPS	>10,000

Assay Characteristics:

K_D (binding affinity):   0.20 nM

B_{max} (receptor number):   166 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [³H]Nitrendipine (70-87 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific DeterminantL   Nifedipine - [1.0 μM]

Reference Compound: Nifedipine

Positive Control: Nifedipine

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the nitrendipine binding site.

Literature Reference:

R. Gould, K. Murphy, and S. Snyder. Tissue Heterogeneity of Calcium Channel Antagonist Binding Sites Labeled by [³H]Nitrendipine. *Molecular Pharmacology.* **25:** 235-241 (1984) with modifications .

Ehlert, F.J., Roeske, W.R., Yamamura, H.I. The Binding of [³H]Nitrendipine to Receptors for Calcuim Channel Antagonists in the Heart, Cerebral Cortex, and Ileum of Rats. *Life Sci.* **30:** 2191-2202 (1982).

CALCIUM CHANNEL, TYPE N
BINDING ASSAY
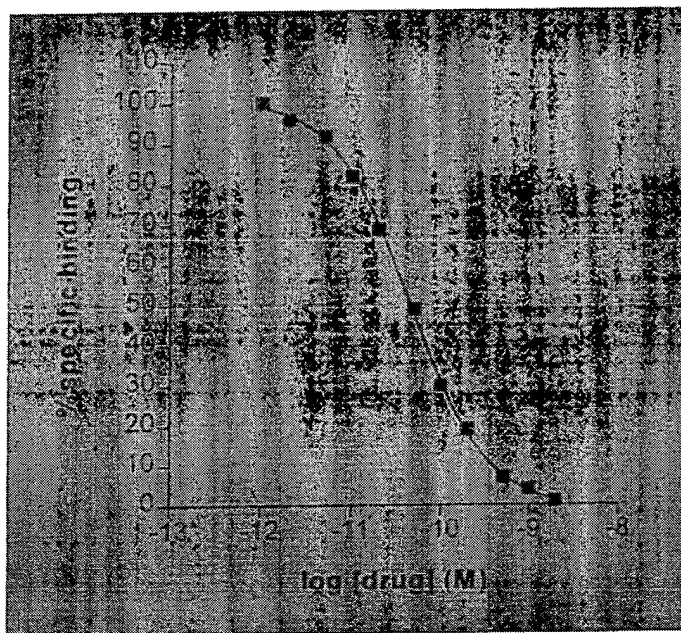
Reference Compounds	Ki (nM)
■ ω-Conotoxin GVIA	0.010
Neomycin sulfate	1500.0
Verapamil	>10,000
Nitrendipine	>10,000
Diltiazem	>10,000
TBOB	>100,000
Charybdotoxin	>100,000
Apamin	>100,000
Saxitoxin	>100,000

Assay Characteristics:

K_D (binding affinity):   0.01 nM

B_{max} (receptor number):   23.0 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [^{125}I] ω-conotoxin (2000 Ci/mmol)
Final ligand concentration - [0.01nM]

Non-specific Determinant: ω-conotoxin GVIA - [100.0 nM]

Reference Compound: ω-conotoxin GVIA

Positive Control: ω-conotoxin GVIA

Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.4) containing 0.2% BSA at 25°C for 30 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the ω-conotoxin binding site.

Literature Reference: Wagner, J., Snowman, A., Biswas, A., Olivera, S. and Snyder, S.H. Omega-Conotoxin GVIA Binding to a High-Affinity Receptor in the Brain: Characterization, Calcium Sensitivity and Solubilization. *Jrnl. Neuroscience.* **8(9):** 3354-3359 (1988) with modifications.

Pullman, L.M., Keith, R.A., LaMonte, D., et al. The Polyamine Spermine Affects ω-Conotoxin Binding and Function at N-Type Voltage-Sensitive Calcium Channels. *Jrnl. Auton. Pharmacol.* **10:** 213-219 (1990).
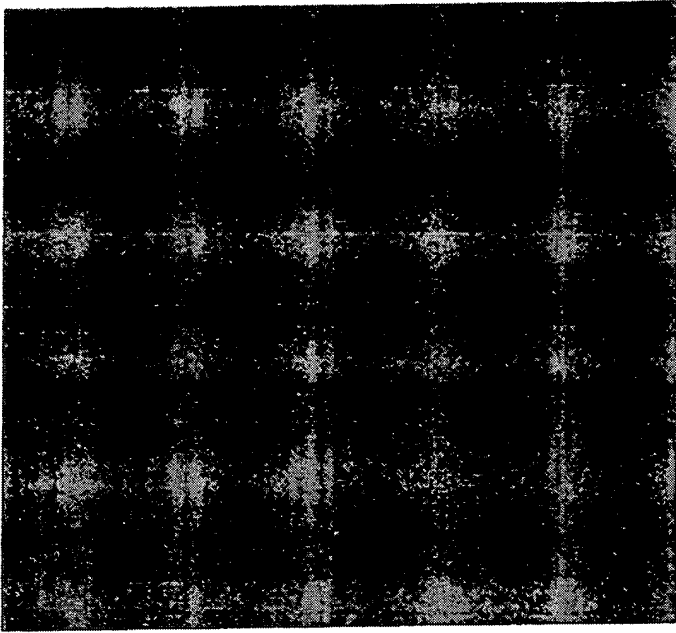
CALCIUM CHANNEL, TYPE L (BENZOTHIAZEPINE SITE)
BINDING ASSAY
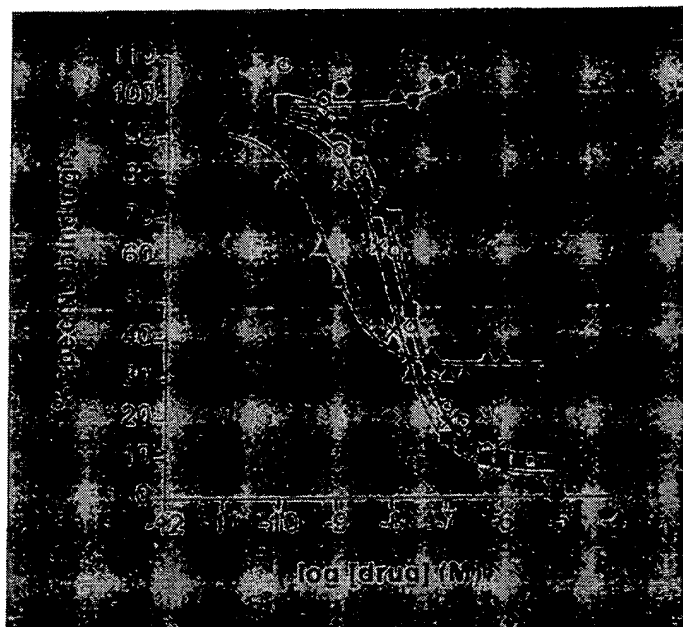
Reference Compounds	Ki (nM)
▲ Nifedipine	1.3
✕ (+/-) MethoxyVerapamil	
(D600)	12.5
○ (+/-) Verapamil	14.4
■ Diltiazem HCl	27.3
● Conotoxin GVIA	>100.0

Assay Characteristics:

K_d (binding affinity):	34 nM
B_{max} (receptor number):	24.2 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [^{3}H]Diltiazem (70-87 Ci/mmol)
Final ligand concentration - [5.0 nM]

Non-specific Determinant: Diltiazem HCl - [10 μM]

Reference Compound: Diltiazem HCl

Positive Control: Diltiazem HCl

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the diltiazem binding site.

Literature Reference:

Schoemaker and Langer. [3H]Diltiazem Binding to Calcium Channel Antagonists Recognition Sites in Rat Cerebral Cortex. *Eur. Jrnl. Pharm.* **111**: 273-277 (1985) with modifications.

CHOLECYSTOKININ, CCK$_A$ (PERIPHERAL)
BINDING ASSAY
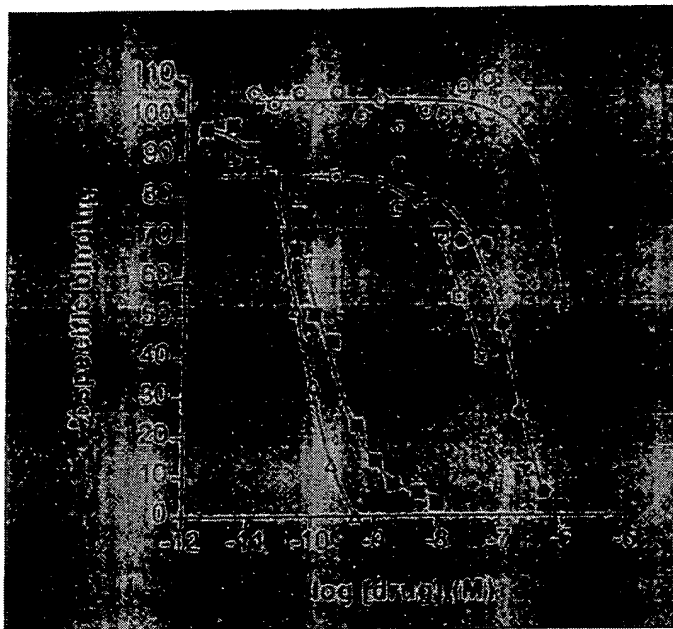
Reference Compounds	Ki (nM)
■ CCK$_{(26-33)}$ (sulfated)	0.076
△ L 364,718	0.029
☐ CCK$_{(26-33)}$ (non-sulfated)	>100.0
● L 365,260	135.0
○ Gastrin	>1,000

Assay Characteristics:

K$_D$ (binding affinity):	31.7 pM
B$_{max}$ (receptor number):	270 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Mouse pancreatic membranes
Radioligand:	[^{125}I]Cholecystokinin (2200Ci/mmol)
	Final ligand concentration - [0.02 nM]
Non-specific Determinant:	Cholecystokinin 8 (26-33) Sulfated - [1.0 µM]
Reference Compound:	Cholecystokinin 8 (26-33) Sulfated
Positive Control:	Cholecystokinin 8 (26-33) Sulfated
Incubation Conditions:	Reactions are carried out in 20 mM HEPES containing 360 mM NaCl, 15 mM KCl, 5 mM MgCl$_2$, 1 mM EGTA, and 0.1% BSA (pH 6.5) at 25°C for 120 minutes. Reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cholecystokinin peripheral binding site.

Literature Reference:

Wennogle, L., Steel, D. and Petrack, B. Characterization of Central CCK Receptors using a Radioiodinated Octapeptide Probe. *Life Sciences.* **36**: 1485-1492 (1985) with modifications.

Innis, R.B., and Snyder, S.H. Distinct Cholecystokinin Receptors in Brain and Pancreas. *Proc. Nat'l Acad. Sci.* **77**: 6239-6243(1980).

CANNABINOID, CB$_2$ (HUMAN RECOMBINANT) BINDING ASSAY
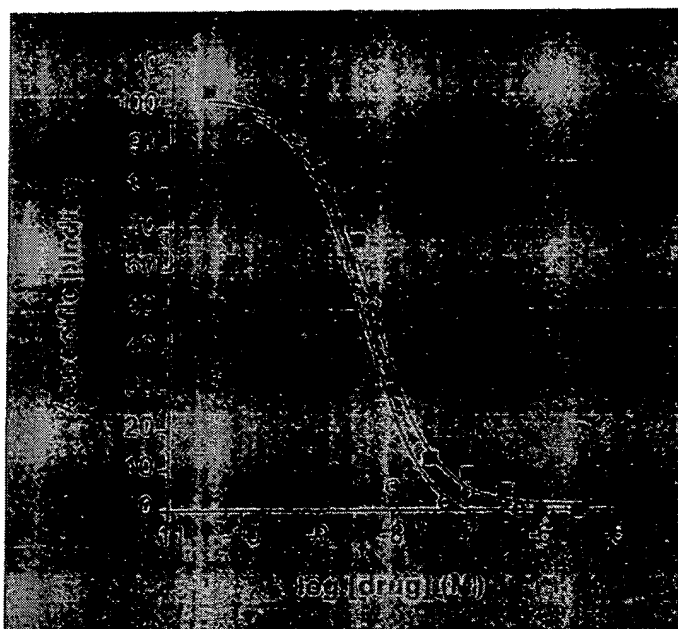
Reference Compound	Ki (nM)
○ HU 210	3.0
■ WIN 55,212-2	7.4

Assay Characteristics:

K$_D$ (binding affinity):　　　　4.1 nM
B$_{max}$ (receptor number):　　　8 pmol/mg protein

Materials and Methods:

Receptor Source:　　　　　Human recombinant expressed in CHO-K1 cells
Radioligand:　　　　　　　[^{3}H]CP 55,940 (100-200 Ci/mmol)
　　　　　　　　　　　　　Final ligand concentration - [0.7 nM]
Non-specific Determinant:　WIN 55,212-2 - [3.0 μM]
Reference Compound:　　　WIN 55,212-2
Positive Control:　　　　　WIN 55,212-2
Incubation Conditions:　　　Reactions are carried out in 25 mM HEPES buffer (pH 7.4) containing 1 mM EDTA, 1 mM MgCl$_2$, and 0.5 mg/ml BSA at 30°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined using liquid scintillation spectrometry and compared to control values in order to ascertain any interactions of test compound with the CB$_2$ binding site.

Literature Reference:　　　Thomas, B.F., et al. Comparative Receptor Binding Analyses of Cannabinoid Agonists and Antagonist. *Jrnl. Pharmacol. Exp. Ther* **285:** 285-292 (1998) with modifications.

　　　　　　　　　　　　　Devane, W.A. *TIPS* **15:** 40-51 561 (1994).

　　　　　　　　　　　　　Felder, C.C. et al. *Molec. Pharmac.* **48:** 443-450 (1995).

GenBank Accession #:　　　X74328

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CHOLINE TRANSPORT
BINDING ASSAY
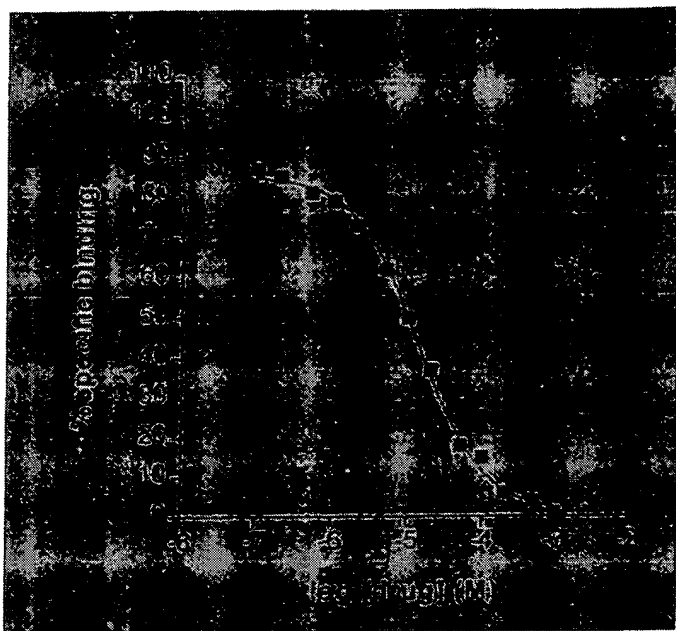
Reference Compounds	Ki (nM)
■ Choline Chloride	7,100

Assay Characteristics:

K_D (binding affinity):	9,500 nM
B_{max} (receptor number):	2.2 pmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]Choline chloride (80-95 Ci/mmol)
	Final ligand concentration - [5.0 nM]
Non-specific Determinant:	Choline chloride - [1 mM]
Reference Compound:	Choline chloride
Positive Control:	Choline chloride
Incubation Conditions:	Reactions are carried out in Krebs-HEPES buffer at 37°C for 10 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the choline uptake site.

Literature Reference:	Atweh, et al. Utilization of Sodium Dependent High Affinity Choline Uptake *In Vitro* as a Measure of the Activity of Cholinergic Neurons *In Vivo*. *Life Sciences*. **17**: 1535-1544 (1975) with modifications.

CHOLECYSTOKININ, CCK$_B$ (CENTRAL)
BINDING ASSAY
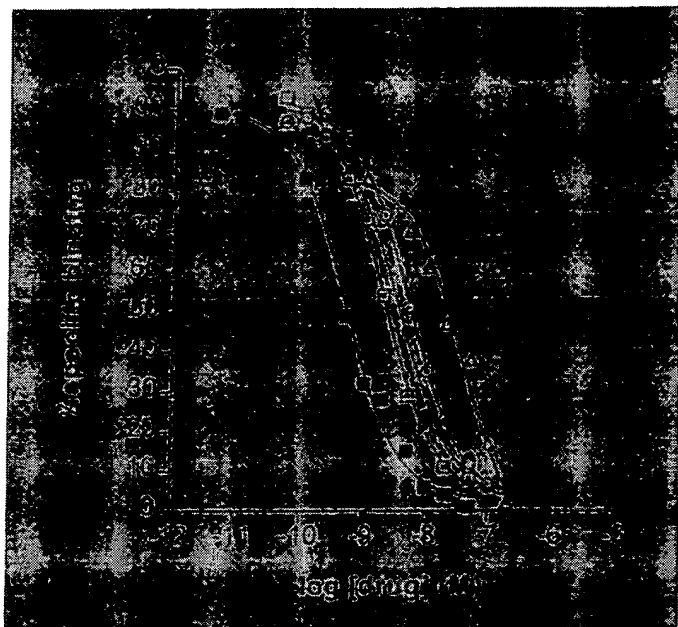
Reference Compounds	Ki (nM)
■ CCK$_{(26-33)}$ (sulfated)	0.5
▫ CCK$_{(26-33)}$ (non-sulfated)	1.9
● L 365,260	3.6
○ Gastrin	4.9
△ L 364,718	34.5

Assay Characteristics:

K$_D$ (binding affinity):   0.2 nM

B$_{max}$ (receptor number):   204 fmol/mg protein

Materials and Methods:

Receptor Source: Mouse forebrain membranes

Radioligand: [^{125}I]Cholecystokinin (2200 Ci/mmol)
Final ligand concentration - [0.02 nM]

Non-specific Determinant: Cholecystokinin 8 (26-33) Sulfated - [1.0 μM]

Reference Compound: Cholecystokinin 8 (26-33) Sulfated

Positive Control: Cholecystokinin 8 (26-33) Sulfated

Incubation Conditions: Reactions are carried out in 20 mM HEPES containing 360 mM NaCl, 15 mM KCl, 5 mM MgCl$_2$, 1 mM EGTA, and 0.1% BSA (pH 6.5) at 25°C for 120 minutes. Reaction is terminated by rapid filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cholecystokinin central binding site.

Literature Reference:

Wennogle, L., Steel, D. and Petrack, B. Characterization of Central CCK Receptors using a Radioiodinated Octapeptide Probe. *Life Sciences.* **36:** 1485-1492 (1985) with modifications.

Sekiguchi, R. and Moroji, T. A Comparative Study on Characterization and Distribution of Cholecystokinin Binding Sites Among the Rat, Mouse, and Guinea Pig Brain. *Brain Res.* **399:** 271-281 (1986).

COMPLEMENT C5a (HUMAN)
BINDING ASSAY
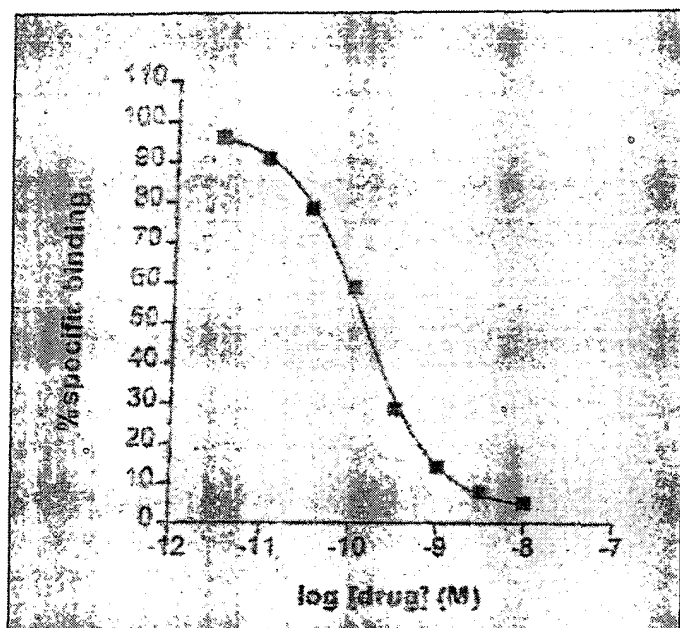
Reference Compound	Ki (nM)
□ rC5a, human	0.2

Assay Characteristics:

K_D (binding affinity):	25 pM
B_{max} (receptor number):	147 fmol/mg protein

Materials and Methods:

Receptor Source:	U937 Cells
Radioligand:	[^{125}I] BH-rC5a, human (2200 Ci/mmol)
	Final ligand concentration - [25.0 pM]
Non-specific Determinant:	rC5a, human - [0.2 µM]
Reference Compound:	rC5a, human
Positive Control:	rC5a, human
Incubation Conditions:	Reactions are carried out in 50 mM HEPES (pH 7.5) buffer containing 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.1% BSA, 0.1 mM PMSF, and 0.1% bacitracin for 120 minutes at 4°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the complement C5a binding site.

Literature Reference:

Johnson, R.J. and Chenoweth, D.E. Synthesis of a New Photoreactive C5a Analog that Permits Identification of the Ligand Binding Component of the Granulocyte C5a Receptor. *Biochem. Biophys. Res. Comm.* **148(3):** 1330-1337 (1987) with modifications.

Zimmerli, W., Reber, A-M., and Dahinden, C.A. The Role of Formylpeptide Receptors, C5a Receptors, and Cytosolic-Free Calcium in Neutrophil Priming. *Jrnl. Infect. Dis.* **161:** 242-249 (1990).

CLOZAPINE
BINDING ASSAY
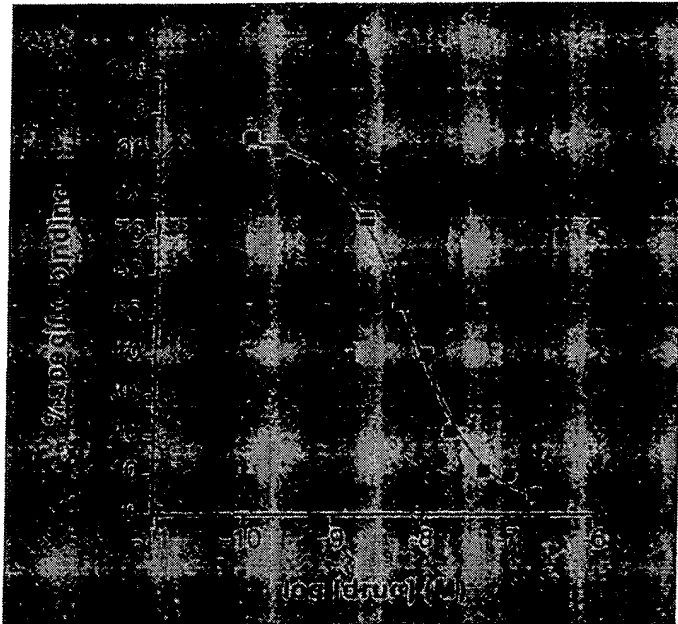
Reference Compounds	Ki (nM)
■ Clozapine	4.8
Fluphenazine	18.0
Raclopride	>1,000
Eticlopride	>1,000

Assay Characteristics:

K_D (binding affinity):	20.0 nM
B_{max} (receptor number):	105 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat striatal membranes
Radioligand:	[^{3}H]Clozapine (80 - 90 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Clozapine - [1.0 μM]
Reference Compound:	Clozapine
Positive Control:	Clozapine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) with 12.5 nM scopolamine and 0.125% BSA at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the clozapine binding site.

Literature Reference:	Unpublished

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DOPAMINE, NON-SELECTIVE
BINDING ASSAY
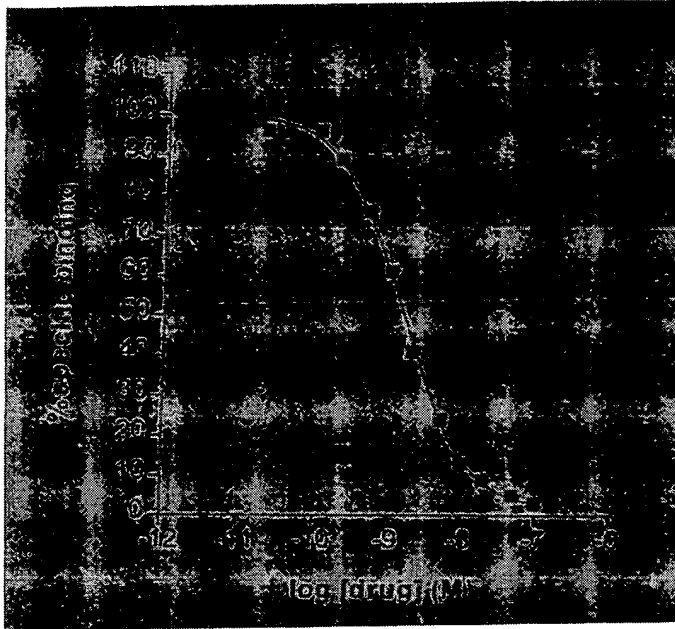
Reference Compounds	Ki (nM)
■ (+/-)-Spiperone	1.7

Assay Characteristics:

K_0 (binding affinity):	0.7 nM
B_{max} (receptor number):	40.5 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Bovine striatal membranes
Radioligand:	[³H]Spiperone (15-25 Ci/mmol)
	Final ligand concentration - [0.3 nM]
Non-specific Determinant:	(+/-) Spiperone - [1.0 μM]
Reference Compound:	(+/-) Spiperone
Positive Control:	(+/-) Spiperone
Incubaton Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, and 1 mM MgCl₂ at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the dopamine binding site.

Literature Reference:

Leysen, Gommereren, et al. Spiperone: A Ligand of Choice for Neuroleptic Receptors (1. Kinetics and Characteristics of *in vitro* Binding). *Biochem. Pharmacol.* **27**: 307-316 (1978) with modifications.

Creese, I., Schneider, R., and Snyder, S.H. [³H]Spiroperidol Labels Dopamine Receptors in Pituitary and Brain. *Eur. Jrnl. Pharmacol.* **46**: 377-381 (1977).

CORTICOTROPIN RELEASING FACTOR (CRF)
BINDING ASSAY
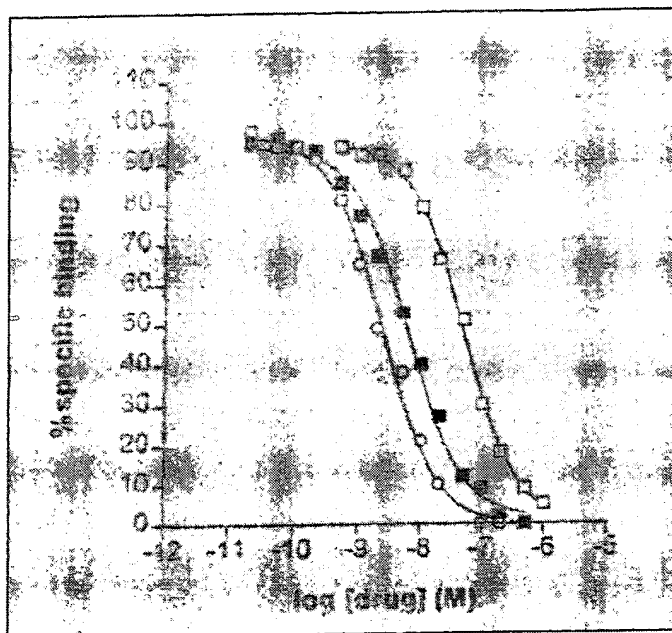
Reference Compounds	Ki (nM)
O oCRF	2.3
□ Tyr⁰-oCRF	3.5
□ α-Helical oCRF$_{(9-41)}$	41.1

Assay Characteristics:

K_D (binding affinity):   4.5 nM

B_{max} (receptor number):   243 fmol/mg protein

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [^{125}I]Tyr-oCRF (2200 Ci/mmol)
Final ligand concentration - [0.1 nM]

Non-specific Determinant: Tyr⁰-oCRF (Corticotropin releasing factor, Tyr⁰-ovine) - [1.0 µM]

Reference Compound: Tyr⁰-oCRF (Corticotropin releasing factor, Tyr⁰-ovine)

Positive Control: Tyr⁰-oCRF (Corticotropin releasing factor, Tyr⁰-ovine)

Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.0) containing 10 mM $MgCl_2$, 2 mM EGTA and 0.3% BSA and 0.12 TIU/ml aprotinin at 25°C for 120 minutes. The reaction is terminated by centrifugation of the assay tubes in a Sorvall centrifuge for 15 minutes at 4°C. After repeat washings, the resulting pellet is saved and placed into tubes. Radioactivity trapped in the tissue pellet is assessed using gamma spectrometry.

Literature Reference:

De Souza, E.B. Corticotropin Releasing Factor Receptors in the Rat Central Nervous System: Characterization and Regional Distribution. *Jrnl. Neuroscience.* **7(1):** 88-100 (1987) with modifications.

De Souza, E.B., Insel, T.B., et al. Corticotropin Releasing Facto Receptors are Widely Distributed within the Rat Central Nervous System: An Autoradiographic Study. *Jrnl. Neuroscience.* **5:** 3189-3203 (1985).

DOPAMINE, D$_1$ (HUMAN RECOMINANT) BINDING ASSAY
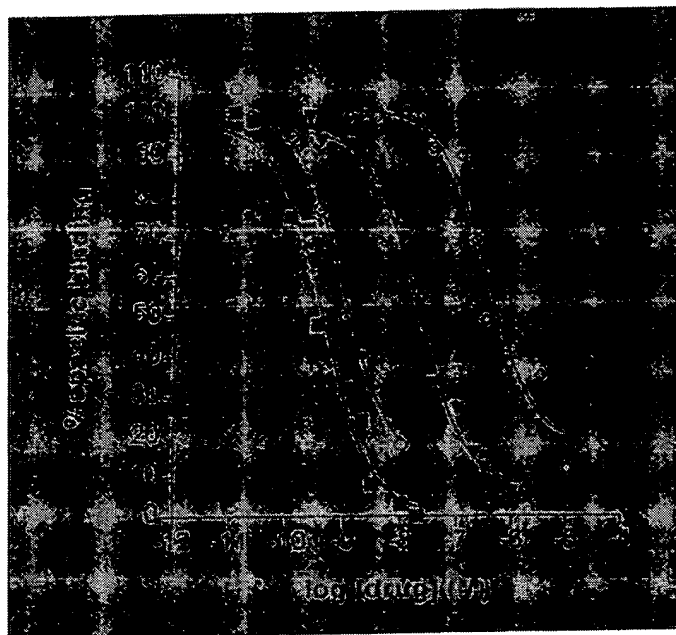
Reference Compound	Ki (nM)
■ R(+)-SCH-23390	0.2
O SKF 83566	0.3
▼ Haldol	18.0
◇ Spiperone	119.8

Assay Characteristics:

K$_D$ (binding affinity):	0.3 nM
B$_{max}$ (receptor number):	68 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in L cells
Radioligand:	[^{3}H]SCH 23390 (70-87 Ci/mmol) Final ligand concentration - [0.5 nM]
Non-specific Determinant:	R(+)-SCH 23390 - [10 uM]
Reference Compound:	R(+)-SCH 23390
Positive Control:	R(+)-SCH 23390
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM MgCl$_2$, 5 mM KCl, 5 mM EDTA and 1.5 mM CaCl$_2$ for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned dopamine - D$_1$ binding site.

Literature Reference:

Jarvis, K.R., Tiberi, M., Silvia, C., Gingrich, J.A. and Caron, M.G. Molecular Cloning, Stable Expression and Desensitization of the Human Dopamine D$_{1b}$/D$_5$ Receptor. *Jrnl. Receptor Research.* **13(1-4):** 573-590 (1993).

Billard, W., Ruperto, V., Crosby, G., et al. Characterization of the Binding of [^{3}H]SCH 23390: a Selective D$_1$ Receptor Antagonist Ligand in Rat Striatum. *Life Sciences.* **35:** 1885-1893 (1984) with modifications.

DOPAMINE, D₁
BINDING ASSAY
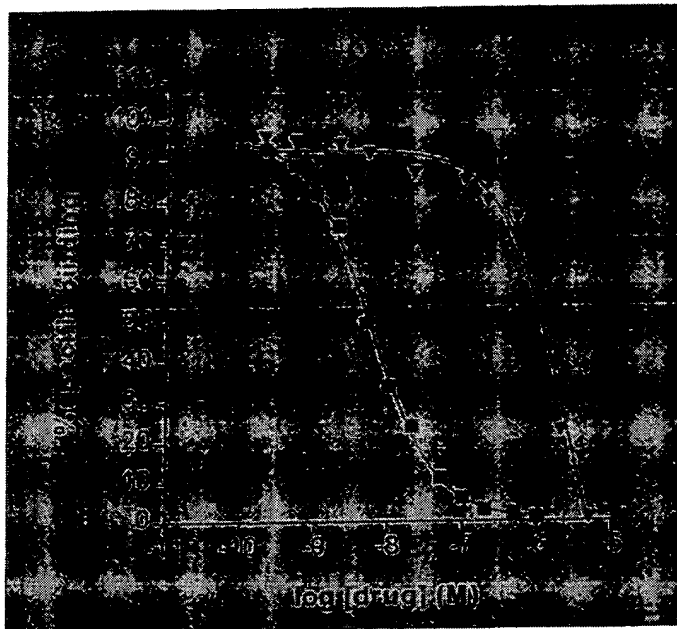
Assay Characteristics:

K_D (binding affinity):   5.3 nM

B_{max} (receptor number):   69 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat striatal membranes

Radioligand: [³H]SCH 23390 (70-87 Ci/mmol)
                          Final concentration - [0.5 nM]

Non-specific Determinant: R(+)-SCH 23390 - [1.0 uM]

Reference Compound: R(+)-SCH 23390

Positive Control: R(+)-SCH 23390

Incubaton Conditions: Reactions are carried out in 50 mM HEPES (pH 7.4) containing 1.0 mM EDTA, 4.0 mM MgSO₄, and 10 µM ketanserin at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the dopamine₁ binding site.

Literature Reference:

Billard, W., Ruperto, V., Crosby, G., et al. Characterization of the Binding of [³H]SCH 23390: a Selective D₁ Receptor Antagonist Ligand in Rat Striatum. *Life Sciences.* **35**: 1885-1893 (1984) with modifications.

Anderson, P.H., Gronvald, F.C., et al. NNC-112, NNC-687, and NNC-756, New Selective and Highly Potent Dopamine D₁ Receptor Antagonists. *Eur. Jrnl. Pharmac.* **219(1)**: 45-52 (1992).

DOPAMINE, $D_{2\ SHORT}$ (HUMAN RECOMBINANT) BINDING ASSAY
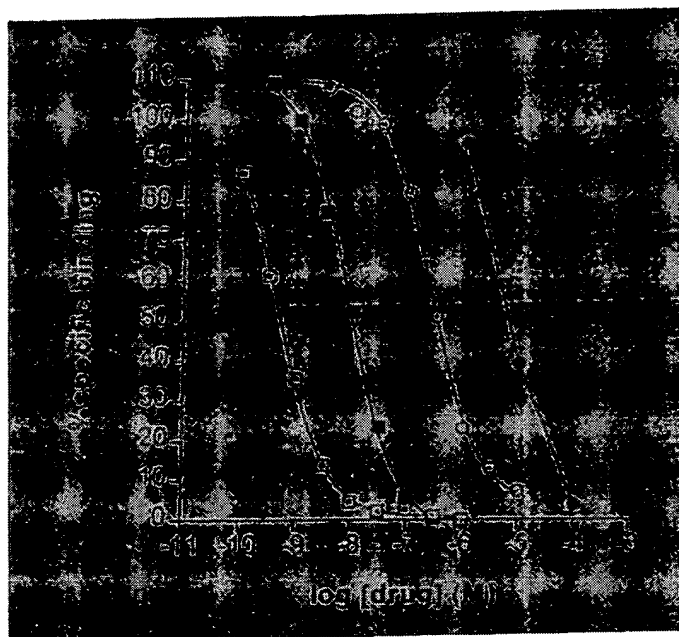
Reference Compounds	Ki (nM)
□ Eticlopride	0.2
■ Haloperidol	2.8
○ Sulpiride	83.8
—●— SCH23390	1,000.0 est.*

* estimated

Assay Characteristics:

K_D (binding affinity):	0.1 nM
B_{max} (receptor number):	1.5 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{3}H]Spiperone (20-60 Ci/mmol)
	Final ligand concentration - [0.2 nM]
Non-specific Determinant:	Haloperidol - [1.0 uM]
Reference Compound:	Haloperidol
Positive Control:	Haloperidol
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned dopamine - D_2 short binding site.

Literature Reference:

Jarvis, K.R., Tiberi, M., Silvia, C., Gingrich, J.A. and Caron, M.G. Molecular Cloning, Stable Expression and Desensitization of the Human Dopamine D_{1b}/D_5 Receptor. *Jrnl. Receptor Research.* **13(1-4):** 573-590 (1993).

Gundlach, A.L., Largent, B.L., and Snyder, S.H. Spiperone: A Novel Ligand for D_2 Dopamine Receptors. *Life Sciences.* **35:** 1981-1988 (1984) with modifications.

GenBank Accession Number: S62137

DOPAMINE, D_2
BINDING ASSAY
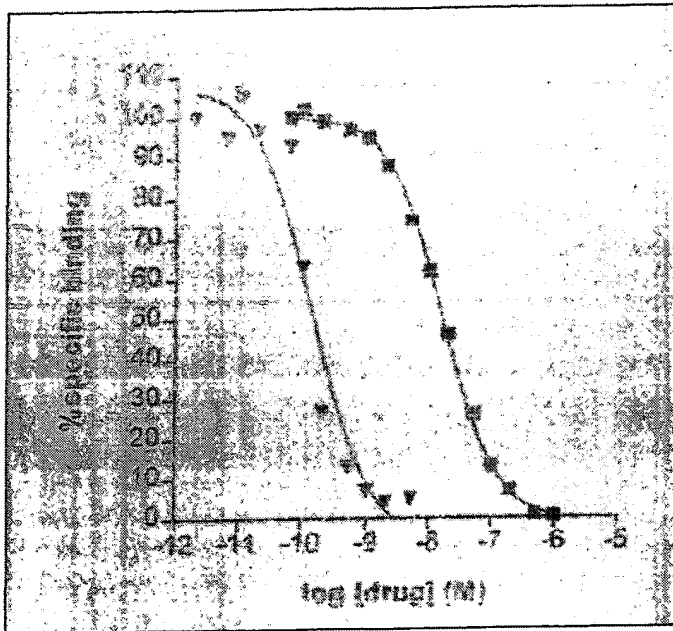
Reference Compounds	Ki (nM)
▽ Spiperone	0.1
Butaclamol	0.7
▣ (+/-)-Sulpiride	5.6
Metoclopramide	10.2
SKF 38393	475.0
R(+)-SCH 23390	503.0

Assay Characteristics:

K_D (binding affinity):	3.0 nM
B_{max} (receptor number):	348 fmol/mg protein

Materials and Methods:

Receptor Source: Rat striatal membranes
Radioligand: [^{3}H]Sulpiride (60-90 Ci/mmol)
Final ligand concentration - [3.0 nM]

Non-specific Determinant: (+/-) Sulpiride - [1.0 μM]
Reference Compound: (+/-) Sulpiride
Positive Control: (+/-) Sulpiride
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.5) containing 100 mM NaCl, 5 mM $MgCl_2$, and 0.02% Ascorbate at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the dopamine$_2$ binding site.

Literature Reference:

Imafuku, J., et al. The Characterization of [^{3}H]Sulpiride Binding Sites in Rat Striatal Membranes. *Brain Research.* **402**: 331-338 (1987) with modifications.

Tsuchihashi, H., Sasaki, T., et al. Binding of [^{3}H]Haloperidol to Dopamine D_2 Receptors in the Rat Striatum. *Jrnl. Pharm.* **44(11)**: 911-914 (1992).

DOPAMINE, $D_{4.2}$ (HUMAN RECOMBINANT)
BINDING ASSAY
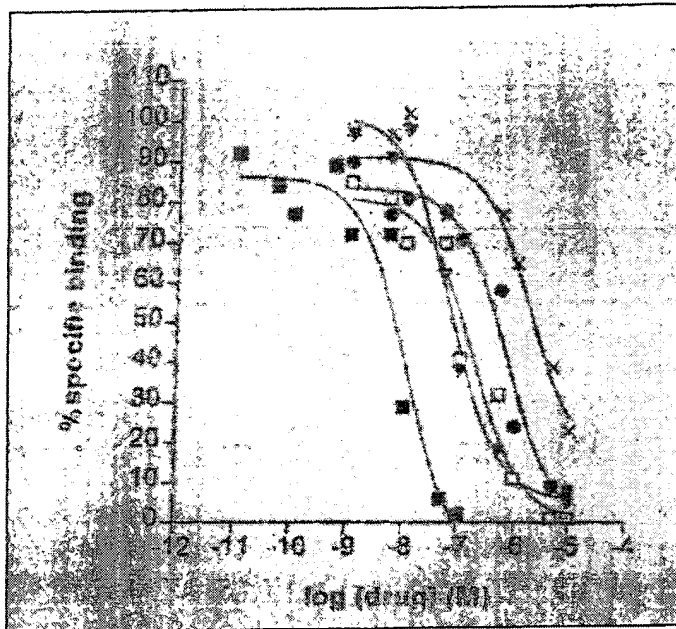
Reference Compounds		Ki (nM)
□	Haldol	6.3
▽	Clozapine	36.4
□	Eticlopride	97.5
○	7OH-DPAT	372.5
×	Sulpiride	994.5

Assay Characteristics:

K_D (binding affinity):   0.25 nM
B_{max} (receptor number):   1.5 pmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO cells
Radioligand: [^{3}H]Spiperone (100-110 Ci/mmol)
                 Final ligand concentration - [0.2 nM]
Non-specific Determinant: Haloperidol (Haldol) - [1.0 uM]
Reference Compound: Haloperidol (Haldol)
Positive Control: Haloperidol (Haldol)
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 120 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA and 5 mM KCl for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned dopamine - $D_{4.2}$ binding site.

Literature Reference: Van Tol et al. Cloning of the Gene for a Human Dopmaine D_4 Receptor with High Affinity for the Antipsychotic Clozapine. *Nature.* **350:** 610 (1991).

Van Tol et al. Multiple Dopmaine D_4 Receptor Variants in th Human Population. *Nature.* **358:** 149 (1992).

Seeman et al. Dopamine D_4 Receptor Bind Inactive (+)-Aporphines, Suggesting Neuroleptic Role. Sulpiride not Stereoselective. *Eur. Jrnl. Pharmac.* **233:** 173 (1993).

DOPAMINE, D₃ (RAT RECOMBINANT)
BINDING ASSAY
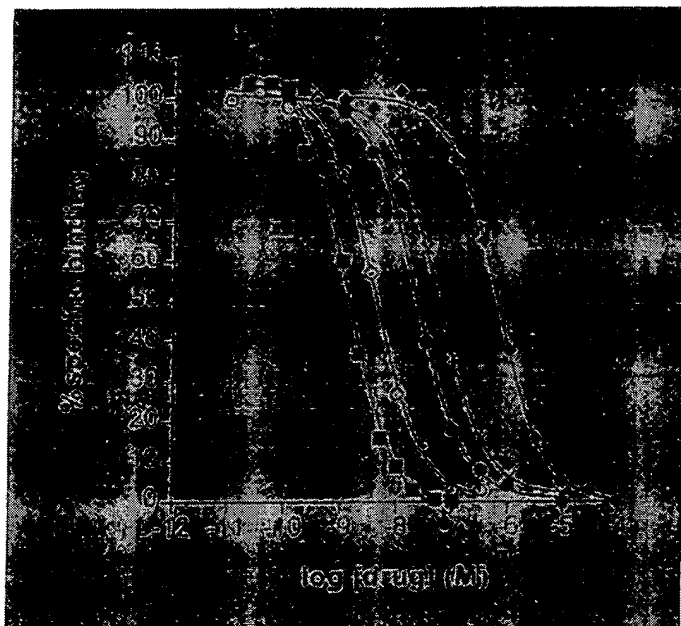
Reference Compounds	Ki (nM)
■ 7-OH-DPAT	0.7
○ Quinpirole	2.1
● Haloperidol	11.6
× Dopamine	55.0
◆ SCH23390	530.0

Assay Characteristics:

K_D (binding affinity): 0.9 nM

B_{max} (receptor number): 24.6 pmol/mg protein

Materials and Methods:

Receptor Source: Rat recombinant D₃ expressed in SF9 cells

Radioligand: [³H]7-OH-DPAT (140-160 Ci/mmol)
Final ligand concentration - [0.75 nM]

Non-specific Determinant: 1.0 μM 7-OH-DPAT

Reference Compound: 7-OH-DPAT

Positive Control: Haloperidol

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM MgCl₂, 5 mM EDTA, 5 mM KCl, 1.5 mM CaCl₂ and 120 mM NaCl at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned dopamine - D₃ binding site.

Literature Reference: Levesque, et al. Identification, Characterization, and Localization of the Dopamine D₃ Receptor in Rat Brain Using 7-[3H]Hydroxy–N,N-di-n-Propyl-2-Aminotetralin. *Proc. Natl. Acad. Sci. USA.* **89:** 8155-8159 (1992) with modifications.
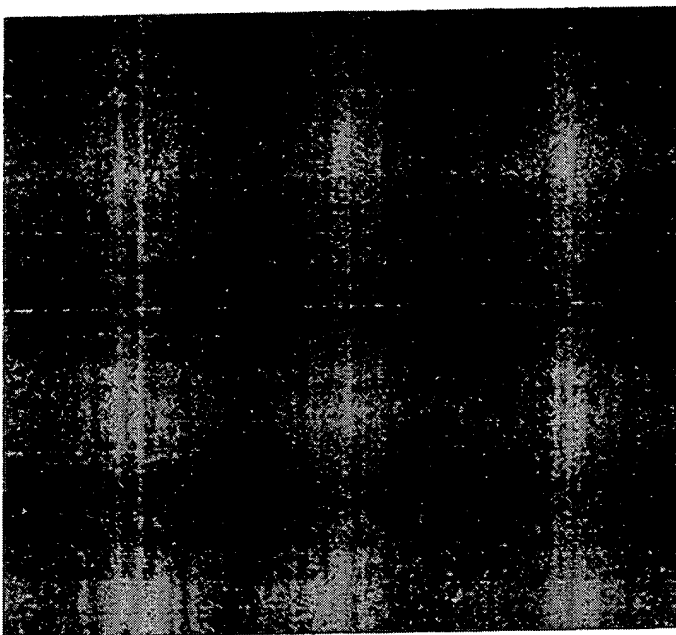
DOPAMINE, $D_{4.4}$ (HUMAN RECOMINANT) BINDING ASSAY
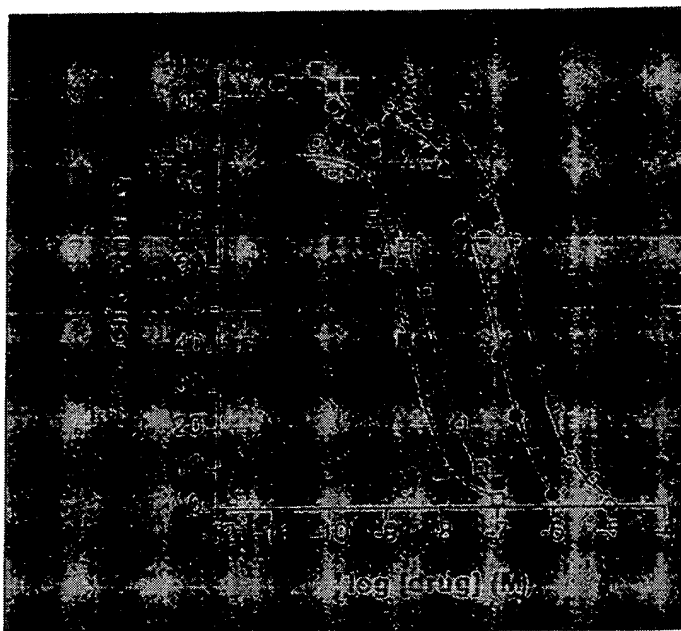
Reference Compound	Ki (nM)
■ Spiperone	0.8
□ Haldol	3.6
● Clozapine	44.6
○ (+)Butaclamol	170.6

Assay Characteristics:

K_D (binding affinity):   0.26 nM

B_{max} (receptor number):   43 pmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO cells

Radioligand: [³H]YM-09151-2 (70-87 Ci/mmol)

Final ligand concentration - [0.3 nM]

Non-specific Determinant: Haloperidol (Haldol) - [1.0 uM]

Reference Compound: Haloperidol (Haldol)

Positive Control: Haloperidol (Haldol)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl and 1.5 mM $CaCl_2$ for 60 minutes at 22°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned dopamine - $D_{4.4}$ binding site.

Literature Reference:

Van Tol et al. Cloning of the Gene for a Human Dopmaine D_4 Receptor with High Affinity for the Antipsychotic Clozapine. *Nature*. **350**: 610 (1991).

Van Tol et al. Multiple Dopmaine D_4 Receptor Variants in th Human Population. *Nature*. **358**: 149 (1992).

Seeman et al. Dopamine D_4 Receptor Bind Inactive (+)-Aporphines, Suggesting Neuroleptic Role. Sulpiride not Stereoselective. *Eur. Jrnl. Pharmac.* **233**: 173 (1993).

DOPAMINE TRANSPORTER
BINDING ASSAY
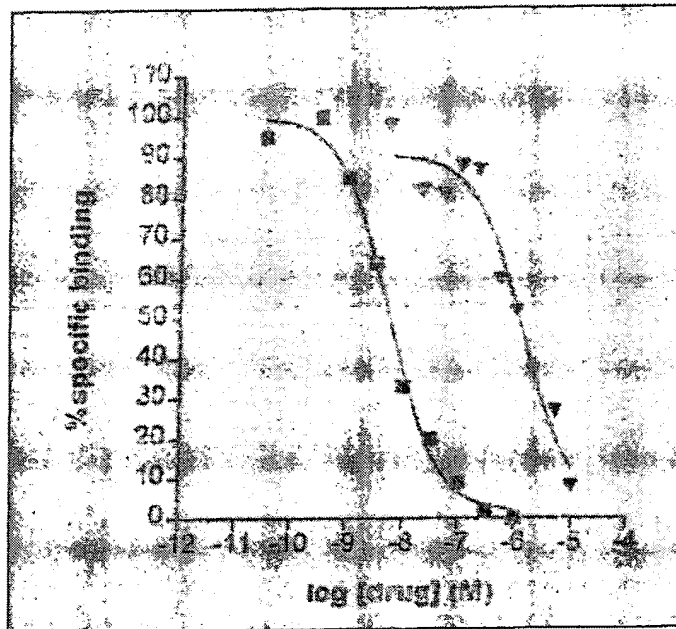
Reference Compounds	Ki (nM)
▫ GBR-12909	7.1
▽ Bupropion	1212.0

Assay Characteristics:

K_D (binding affinity):	28.0 nM
B_{max} (receptor number):	113 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Guinea pig striatal membranes
Radioligand:	[^{3}H]WIN,35,428 (60-87 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	1 uM GBR-12909
Reference Compound:	GBR-12909
Positive Control:	GBR-12909
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 120 mM NaCl 0-4^{0}C for 2 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the dopamine uptake site.

Literature Reference:

Madras, et al. Cocaine Receptors Labeled by [^{3}H]2Beta-Carbomethoxy-3-Beta-(4-Fluorophenyl)topane. *Mol. Pharmacol.* **36**: 518-524 (1989) with modifications.

Javitch, J. J., Blaustein, R. O., and Snyder, S. H. [^{3}H]Mazindol Binding Associated with Neuronal Dopamine and Norepinephrine Uptake Sites. *Mol. Pharmacol.* **26**: 35-44 (1984).

DOPAMINE, D₅ (HUMAN RECOMINANT)
BINDING ASSAY
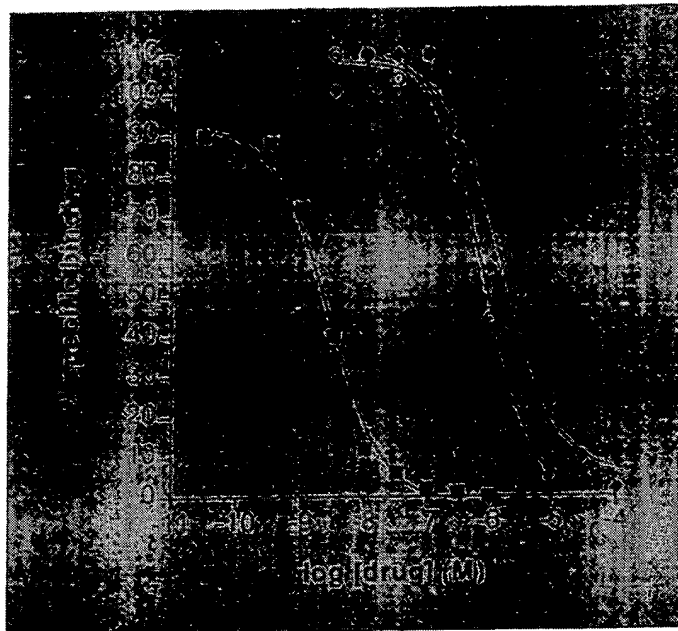
Reference Compounds	Ki (nM)
■ SCH 23390	0.9
○ Clozapine	278.0
◆ Dopamine	1230.0

Assay Characteristics:

K_D (binding affinity):	0.8 nM
B_{max} (receptor number):	14 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in HEK cells
Radioligand:	[³H]SCH 23390 (70-87 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	R(+)-SCH 23390 - [1.0 µM]
Reference Compound:	R(+)-SCH 23390
Positive Control:	R(+)-SCH 23390
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 150 mM NaCl, 0.025% ascorbic acid and 0.001% BSA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned dopamine - D₅ binding site.

Literature Reference:	Grandy et al, 1991
	Sunahara et al, 1991
	Weinshank et al, 1991

GenBank Accession Number:	M67439

ENDOTHELIN$_B$ (HUMAN RECOMBINANT) BINDING ASSAY
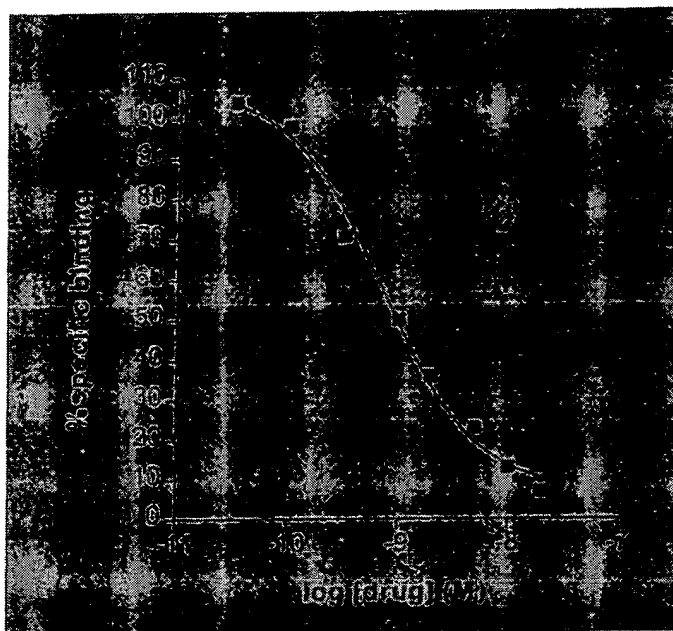
Reference Compound .... Ki. (nM)

■ Endothelin-1   0.1

Assay Characteristics:

K$_D$ (binding affinity):   0.025 nM

B$_{max}$ (receptor number):

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{125}I]Endothelin-1 (2000 Ci/mmol)
	Final ligand concentration - [0.025 nM]
Non-specific Determinant:	Endothelin-1 - [0.1 uM]
Reference Compound:	Endothelin-1
Positive Control:	Endothelin-1
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 0.5 mM CaCl$_2$, 0.05% Tween-20 and 0.1% BSA at 37°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the endothelin B binding site.

Literature Reference: Bolger, G.T., Liard, F., Krogsrud, R., et al. Tissue Specificity of Endothelin Binding Sites. *Jrnl. Cardiovas. Pharmacol.* **16:** 367-375 (1990) with modifications.

ENDOTHELIN$_A$ (HUMAN RECOMBINANT)
BINDING ASSAY
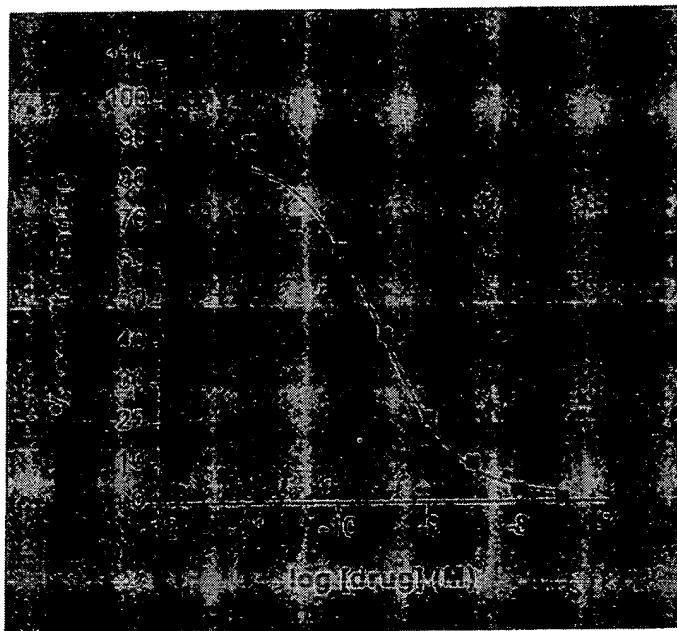
Reference Compound	Ki (nM)
■ Endothelin-1	0.1

Assay Characteristics:

K_D (binding affinity):	0.11 nM
B_{max} (receptor number):	

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{125}I]Endothelin-1 (2000 Ci/mmol)
	Final ligand concentration - [0.025 nM]
Non-specific Determinant:	Endothelin-1 - [0.1 uM]
Reference Compound:	Endothelin-1
Positive Control:	Endothelin-1
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 0.5 mM CaCl$_2$, 0.05% Tween 20 and 0.1% BSA at 37°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the endothelin A binding site.

Literature Reference:	Bolger, G.T., Liard, F., Krogsrud, R., et al. Tissue Specificity of Endothelin Binding Sites. *Jrnl. Cardiovas. Pharmacol.* **16**: 367-375 (1990) with modifications.

ESTROGEN
BINDING ASSAY
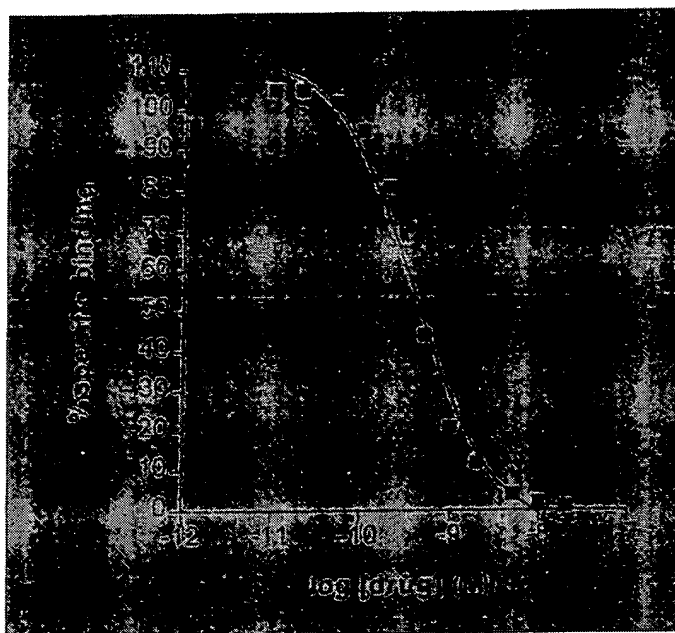
Reference Compounds	Ki (nM)
Diethylstilbestrol	0.04
■ 17β, Estradiol	0.10
Testosterone	400.0
Progesterone	10,000

Assay Characteristics:

K_D (binding affinity):   0.5 nM

B_{max} (receptor number):   0.26 fmol/mg protein

Materials and Methods:

Receptor Source: Bovine uterine membranes

Radioligand: $[^{125}I]3,17β$-Estradiol, 16α (2200 Ci/mmol)

Final ligand concentration - [0.1 nM]

Non-specific Determinant: 17β, Estradiol - [0.1 μM]

Reference Compound: 17β, Estradiol

Positive Control: 17β, Estradiol

Incubation Conditions: Reactions are carried out in 10 mM TRIS-HCl (pH 7.4 containing 1.5 mM EDTA, 1.0 mM DTT, and 25 mM sodium molybdate at 0-4°C for 18 hours. The reaction is terminated by the addition of dextran coated charcoal and incubated for 20 minutes at 0-4°C. The reaction mixtures are centrifuged and the radioactivity bound in the supernatant is assessed and compared to control values in order to ascertain any interactions of test compound with the estradiol binding site.

Literature Reference: Haji, et al. Age-Related Changes in the Concentrations of Cytosol Receptors for Sex Steroids in the Hypothalamus and Pituitary Gland of the Rat. *Brain Research.* **204:** 373-386 (1980) with modifications.

O'Keefe, J.A. and Handa, R.J. Transient Elevation of Estrogen Receptors in the Neonatal Rat Hippocampus. *Develop. Brain Res.* **57:** 119-127 (1990).

EPIDERMAL GROWTH FACTOR (EGF)
BINDING ASSAY
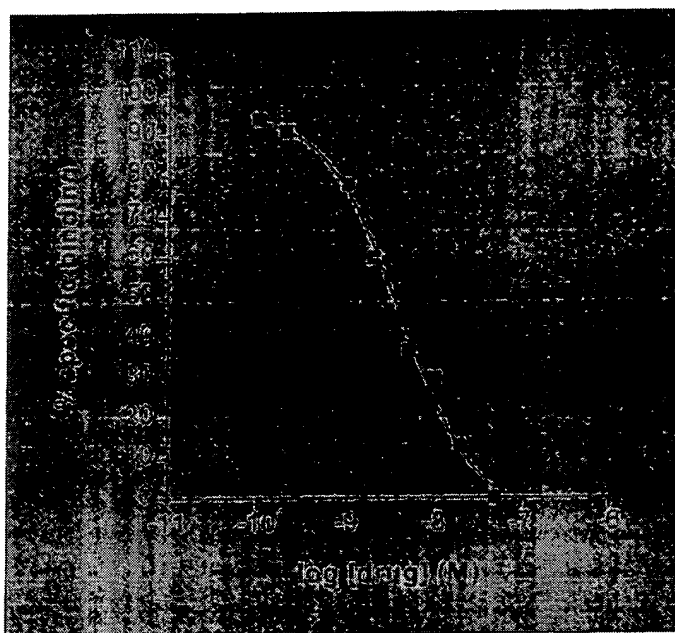
Reference Compounds	Ki (nM)
■ EGF	2.8

Assay Characteristics:

K_D (binding affinity):   1.04 nM

B_{max} (receptor number):   43.0 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat liver membranes
Radioligand:	[^{125}I]Epidermal growth factor (150-200 Ci/ug)
	Final ligand concentration - [0.36 nM]
Non-specific Determinant:	Epidermal growth factor (EGF) - [100 nM]
Reference Compound:	Epidermal growth factor (EGF)
Positive Control:	Epidermal growth factor (EGF)
Incubation Conditions:	Reactions are carried out in 20 mM HEPES (pH 7.4) containing 0.1% BSA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the EGF binding site.

Literature Reference:

Mukku, V.R. Regulation of EGF Receptor Levels by Thyroid Hormone. *Jrnl. Biol. Chem.* **259**: 6543-6546 (1984) wit modifications.

Duh, Q-Y, Siperstein, A.E., Miller, R.A. et al. Epidermal Growth Factor Receptors and Adenylate Cyclase Activity in Human Thyroid Tissues. *World J. Surgery.* **14**: 410-418 (1990).

Lokeshwar, V. B., Huang, S.S., and Huang, J.S. Protamine Enhances EGF-Stimulated Mitogenesis by Increasing Cell Surface EGF Receptor Number. *Jrnl. Biol. Chem.* **264(32)**: 19318-19326 (1989).

GABA$_A$, BENZODIAZEPINE (CENTRAL) SITE BINDING ASSAY
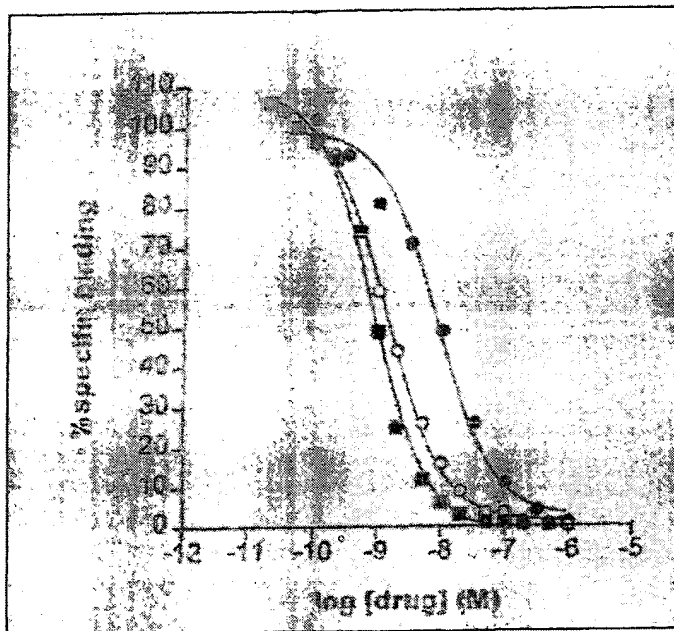
Reference Compounds	Ki (nM)
Ro 15 1788	0.9
Clonazepam	1.0
Lorazepam	4.7
Diazepam	5.6
Ethyl-β-Carboline	6.5

Assay Characteristics:

K_D (binding affinity):	1.4 nM
B_{max} (receptor number):	200 fmol/mg protein

Materials and Methods:

Receptor Source:	Bovine cortical membranes
Radioligand:	[^{3}H]Flunitrazepam (50-100 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specifc Determinant:	Ro15-1788 - [0.5 µM]
Reference Compound:	Ro15-1788
Positive Control:	Ro15-1788
Incubation Conditions:	Reactions are carried out in 10 mM Na-KPO$_4$ (pH 7.7) at 0-4°C for 45 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the benzodiazepine (central) binding site.

Literature Reference:

Sweetnam, P. M. and Tallman, J.F. Regional Differences in Brain Benzodiazepine Receptor Carbohydrates. *Mol. Pharmacol.* **29:** 299-306 (1986) with modifications.

Zarkovsky, A.M. Bicuculline - Sensitive and Insensitive Effects of THIP on the Binding of [^{3}H]Flunitrazepam. *Neuropharmacol.* **26(7A):** 737-741 (1987).

GABA$_A$, AGONIST SITE
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Muscimol	4.4
Isoguvacine	9.5
▼ GABA	23.1
THIP	25.1

Assay Characteristics:

K_D (binding affinity):	370 nM
B_{max} (receptor number):	0.7 pmol/mg protein

Materials and Methods:

Receptor Source:	Bovine cerebellar membranes
Radioligand:	[^{3}H]GABA (70-90 Ci/mmol)
	Final ligand concentration - [5.0 nM]
Non-specific Determinant:	GABA - [1.0 uM]
Reference Compound:	GABA
Positive Control:	GABA
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the GABA$_A$ receptor.

Literature Reference:

Enna, S., et al. Stereospecificity and Structure-Activity Requirements of GABA Receptor Binding in Rat Brain. *Brain Research.* **124**: 185-190 (1977) with modifications.

Falch, E., Hedegaard, A., et al. Comparative Stereostructure - Activity Studies on GABA$_A$ and GABA$_B$ Receptor Sites and GAB Uptake using Rat Brain Membrane Preparations. *Jrnl. Neurochem.* **47(3)**: 898-903 (1986).

GABA_B
BINDING ASSAY
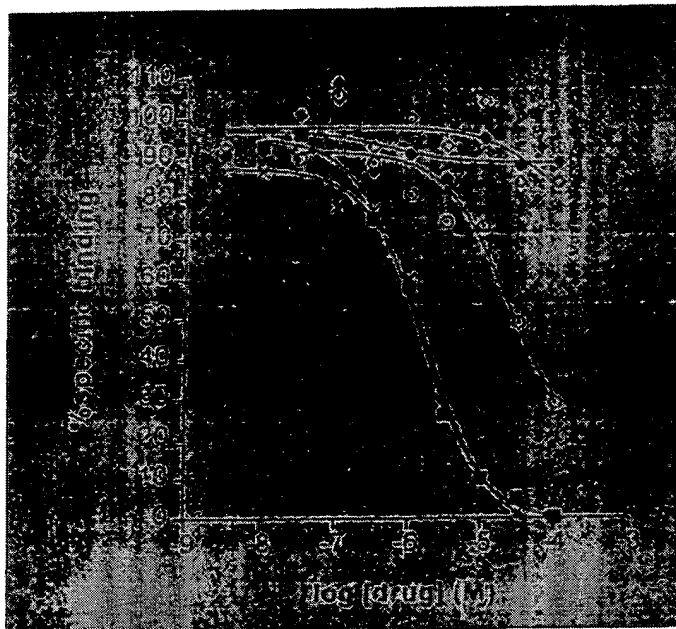
Reference Compounds	Ki (nM)
■ (+/-)Baclofen	761
× GABA	1,069
○ 5-Aminovaleric acid	15,500
♦ THIP	>100,000
◇ Isoguvacine	>100,000

Assay Characteristics:

K_D (binding affinity):	1.2 nM
B_{max} (receptor number):	304 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]CGP (40 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	(+/-)-Baclofen - [100 μM]
Reference Compound:	(+/-)-Baclofen
Positive Control:	(+/-)-Baclofen

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 2.5 mM $CaCl_2$ at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. The amount of radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the GABA_B binding site.

Literature Reference:

Scherer, R.A., Ferkany, J.W., and Enna, S.J. Evidence for Pharmacologically Distinct Subsets of GABA_B Receptors. *Brain Research Bulletin*. **21:** 439-443(1988) with modifications.

Bittiger et al. [^{3}H]CGP54626A: A Potent Antagonist Radioligand for GABA_B Receptors. Pharmacol. Comm. **223:** (1992) with modiifications.

GABA$_A$, CHLORIDE CHANNEL, TBOB SITE
BINDING ASSAY
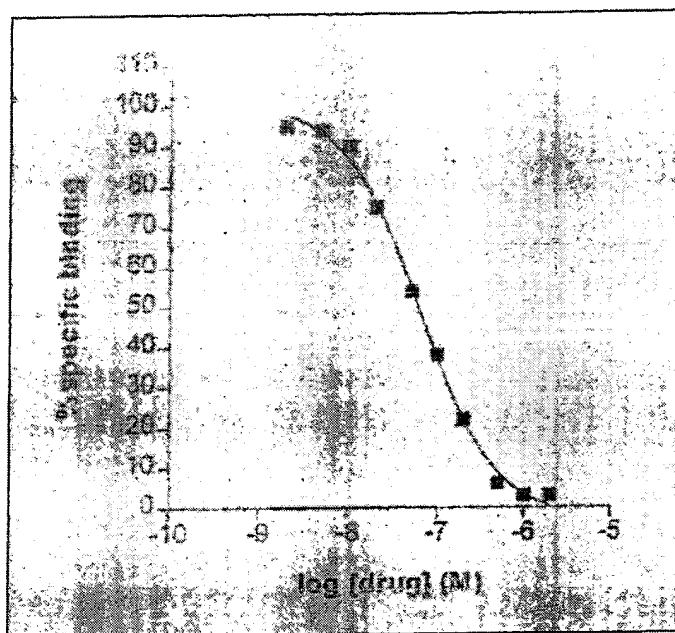
Reference Compounds	Ki (nM)
□ TBPS	48.3
Diltiazem	>10,000
Saxitoxin	>10,000
Charybdotoxin	>10,000

Assay Characteristics:

K$_D$ (binding affinity):   45 nM

B$_{max}$ (receptor number):   116.7 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [^{3}H]TBOB (20-60 Ci/mmol)

Final ligand concentration - [20 nM]

Non-specific Determinant: T-butylbicyclophosphorothionate (TBPS) - [10 μM]

Reference Compound: T-butylbicyclophosphorothionate (TBPS)

Positive Control: T-butylbicyclophosphorothionate (TBPS)

Incubation Conditions: Reactions are carried out in 20 mM NaKPO$_4$/500 mM NaCl (pH 7.5) at 25°C for 75 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the TBOB binding site.

Literature Reference:

Lawrence, L., Palmer, C., Gee, K., Wang, Yamamura, H. and Casida, J. T[^{3}H]butylbicycloorthobenzoate: A New Radioligand Probe for the Gamma-Aminobutyric Acid-Regulated Chloride Ionophore. *Jrnl. Neurochem.* **45(3):** 798-804 (1986) with modifications.

Cole, L.M., Lawrence, L.J., and Casida, J.E. Similar Properties of [^{35}S]t-butylbicyclophosphorothionate Receptor and Coupled Components of the GABA Receptor-Ionophore Complex in Brains of Human, Cow, Rat, Chicken, and Fish. *Life Sci.* **35:** 1755-1762 (1984).

GALANIN
BINDING ASSAY
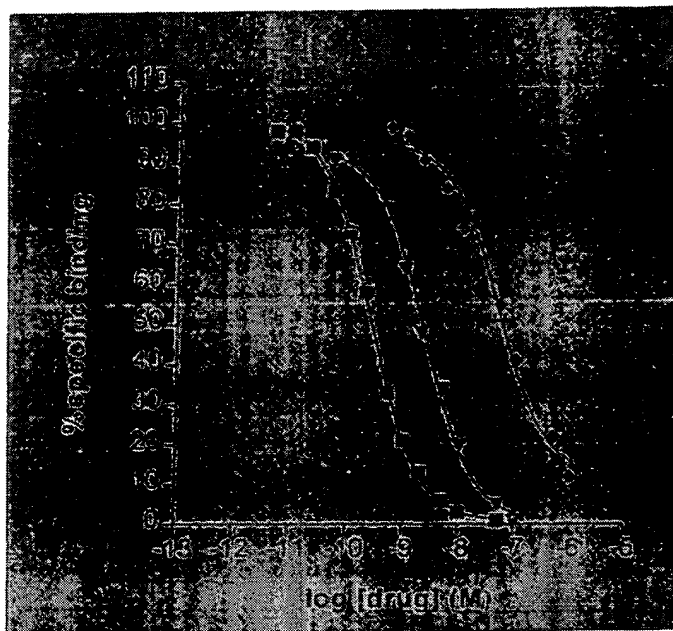
Reference Compounds	Ki (nM)
■ Galanin (porcine)	0.2
▼ Galantide, Galanin Antagonist	2.0
◆ Galanin$_{1-16}$ Agonist	50.0
VIP	>1,000
Somatostatin	>1,000
Cholecystokinin	>1,000

Assay Characteristics:

K_D (binding affinity):	0.1 nM
B_{max} (receptor number):	4.9 fmol/mg tissue (wet weight)

Materials and Methods:

|	
Receptor Source:	Rat brain membranes
Radioligand:	[^{125}I]-Galanin (porcine) (2200 Ci/mmol)
	Final ligand concentration - [0.07 nM]
Non-specific Determinant:	Galanin (porcine) - [100 nM]
Reference Compound:	Galanin (porcine)
Positive Control:	Galanin (porcine)
Incubation Conditions:	Reactions are carried out in 20 mM HEPES (pH 7.5) containing 2% BSA, 1 mg/ml bacitracin, 5 µg/ml leupeptin, and 5.0 µg/ml chymostatin for 2 hours at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the galanin binding site.

Literature Reference:

Servin, A., Amiranoff, B., Rouyer-Fessard, C., Tatemoto, K., and Laburthe, M. Identification and Molecular Characterization of Galanin Receptor Sites in Rat Brain. *Biochem. Biophy Res. Commun.* **114(1):** 298-306 (1987) with modifications.

Skofitsch, G., et al. Autoradiographic Distribution of ^{125}I-Galanin Binding Sites in the Rat Central Nervous System. *Peptides.* **7:** 1029-1042 (1986).

GABA TRANSPORT
BINDING ASSAY

Reference Compounds	Ki (nM)
□ Nipecotic Acid	6,190
△ GABA	26,000

Assay Characteristics:

K_D (binding affinity):   18,000 nM

B_{max} (receptor number):   780 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]GABA (70-80 Ci/mmol)
	Final ligand concentration - [3.6 nM]
Non-specific Determinant:	Nipecotic acid - [1.0 mM]
Reference Compound:	Nipecotic acid
Positive Control:	Nipecotic acid
Incubation Conditions:	Reactions are carried out in KREBS-HEPES (pH 7.4) buffer at 37°C for 3 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the GABA uptake site.

Literature Reference:

Karbon, E. W., Enna, S. J., and Ferkany, J.W. Biochemical and Behavioral Studies Following Subchronic Administration of GABA Uptake Inhibitors in Mice. *Neuropharmacology.* **30:** 1187-1192 (1991).

Falch, E., Hedegaard, A., et al. Comparative Stereostructure - Activity Studies on GABA$_A$ and GABA$_B$ Receptor Sites and GAB Uptake using Rat Brain Membranes. *Jrnl. Neurochem.* **47(3):** 898-903 (1986).

GLUTAMATE, AMPA SITE
BINDING ASSAY
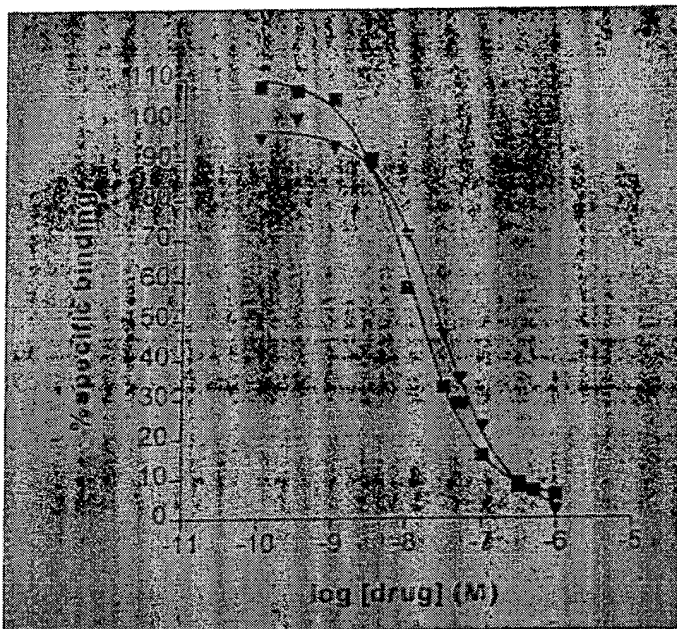
Reference Compounds	Ki (nM)
■ Quisqualic Acid	15.8
▼ AMPA HBr	22.3
L-Glutamate	190.0
CNQX	299.0
NMDA	10,000
Kainic Acid	33,000

Assay Characteristics:

K_D (binding affinity):	28.0 nM
B_{max} (receptor number):	71 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]AMPA (40-70 Ci/mmol)
	Final concentration - [5.0 nM]
Non-specific Determinant:	AMPA - [100 µM]
Reference Compound:	AMPA
Positive Control:	AMPA
Incubation Conditions:	Reactions are carried out in 10 mM K_2HPO_4/100 mM KSCN (pH 7.5) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto filters is determined and compared to control values in order to ascertain any interactions of test compound with the AMPA binding site.

Literature Reference:

Murphy, et al. Characterization of Quisqualate Recognition Sites in Rat Brain Tissue Using [^{3}H]Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic Acid and a Filtration Assay. Neurochem. Res. **12**: 775-781 (1987) with modifications.

Morgan, R.C., et al. Binding of [^{3}H]AMPA to Non Chaotrope, Non-Detergent Treated Rat Synaptic Membranes: Characteristics and Lack of Effect of Barbiturates. Neurochem. Int. **18(1)**: 75-84 (1991).

GLUCOCORTICOID
BINDING ASSAY
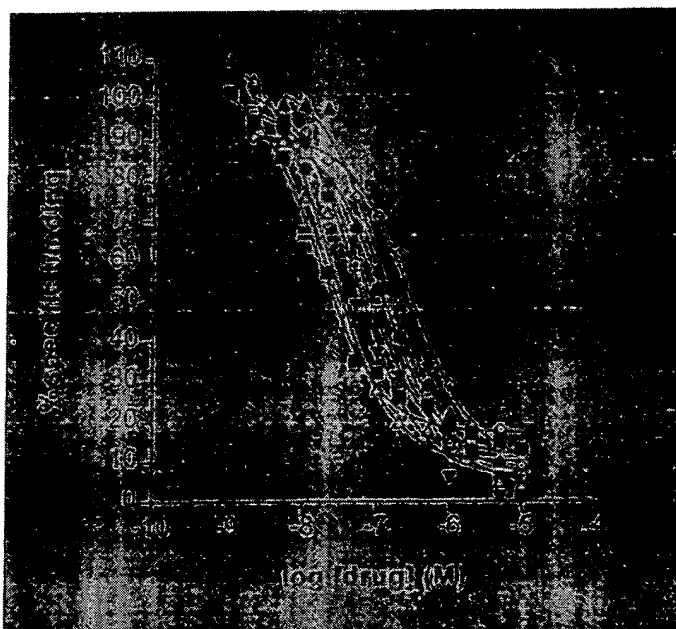
Reference Compounds	Ki (nM)
■ Triamcinolone acetonide	11.3
△ Dexamethosone	15.8
▼ Corticosterone	38.6
□ Prednisone	50.9
▲ Hydrocortisone	98.4
● Progesterone	101.5
◇ Aldosterone	221.0

Assay Characteristics:

K_D (binding affinity):	5.6 nM
B_{max} (receptor number):	73.3 fmol/mg protein

Materials and Methods:

Receptor Source:	Whole rat brain
Radioligand:	[6,7-^{3}H]Triamcinolone acetonide (30-50 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Triamcinolone acetonide - [10 μM]
Reference Compound:	Triamcinolone acetonide
Positive Control:	Triamcinolone acetonide
Incubation Conditions:	Reactions are carried out in 50 mM KH_2PO_4 (pH 7.4) containing 10 mM sodium molybdate and 10 mM α-monothioglycerol at 0°C for 16 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glucocorticoid binding site.

Literature Reference:	Da Han et al. Binding of [^{3}H]Triamcinolone Acetonide to Glucorcorticoid Receptors in Brain Cytosol Fractions of Rats with Intact Adrenals. *Neurochem. Int.* **24**: 339-348 (1994) with modifications.

GLUTAMATE, KAINATE SITE
BINDING ASSAY
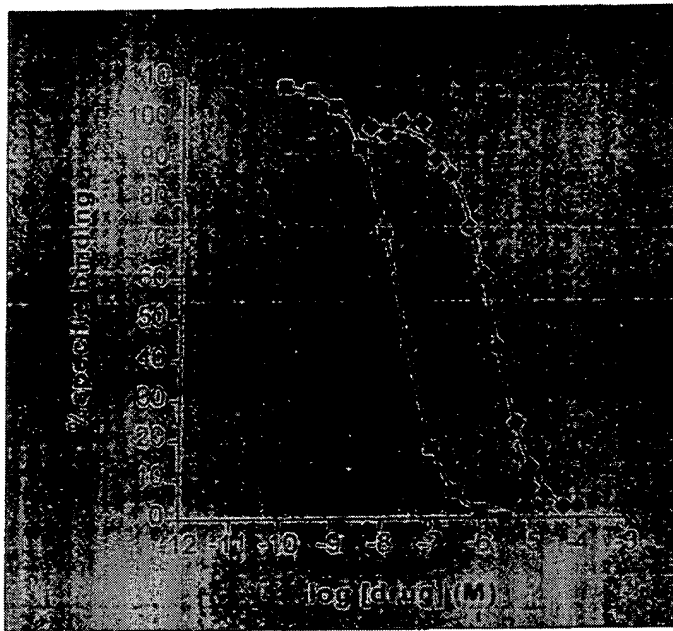
Reference Compounds	Ki (nM)
■ Kainic Acid	9.2
L-Glutamat	220.0
◆ Kainic Acid Dimethyl Ester	914.0
NMDA	>200,000
AMPA	>200,000

Assay Characteristics:

K_d (binding affinity):	16.0 nM
B_{max} (receptor number):	400 fmol/mg protein

Materials and Methods:

|	
Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Kainic acid (30-60 Ci/mmol)
	Final ligand concentration - [10.0 nM]
Non-specific Determinant:	Kainic acid - [100 uM]
Reference Compound:	Kainic acid
Positive Control:	Kainic acid
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl buffer (pH 7.1) at 2°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the kainic acid binding site.

Literature Reference:

London, E. and Coyle, J. Specific Binding of [^{3}H]Kainic Acid to Receptor Sites in Rat Brain. *Mol. Pharmacol.* **15**: 492-505 (1979) with modifications.

Hall, R.A., Kessler, M., and Lynch, G. Kainate Binding to the AMPA Receptor in Rat Brain. *Neurochemical Research.* **19(6)**: 777-782 (1994).

GLUTAMATE, CHLORIDE DEPENDENT SITE
BINDING ASSAY
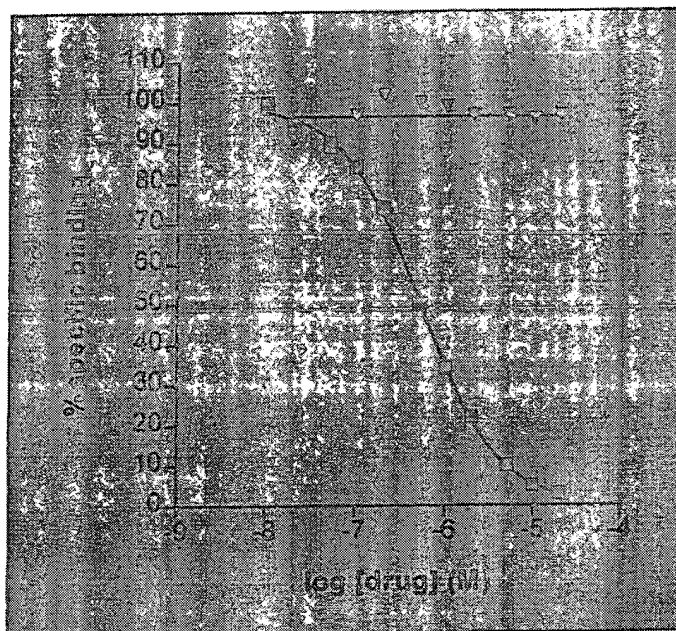
Reference Compounds	Ki (nM)
□ L-Glutamate	761
▽ NMDA	>10,000

Assay Characteristics:

K_D (binding affinity):   1,134 nM

B_{max} (receptor number):   25.6 fmol/mg tissue (wet weight)
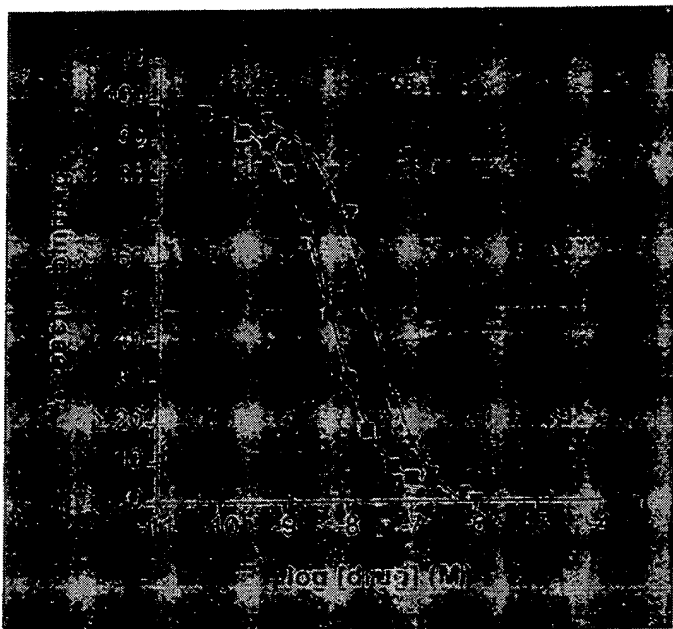
Materials and Methods:

Receptor Source: Rat cerebellar membranes

Radioligand: [³H]Glutamate (40-80 Ci/mmol)

Final ligand concentration - [200 nM]

Non-specific Determinant: L-Glutamic acid - [1.0 mM]

Reference Compound: L-Glutamic acid

Positive Control: L-Glutamic acid

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glutamate binding site.

Literature Reference: Slevin, J., Collins, J., Lindsley, K. and Coyle, J. T. Specific Binding of [³H]-L-Glutamate to Cerebellar Membranes: Evidence for Recognition Site Heterogeneity. *Brain Research.* **249:** 353360 (1982) with modifications.

Cha, J-H. J., Makowiec, R. L., Penney, J. B., and Young, A. B. L-[³H]Glutamate Labels the Metabotropic Excitatory Amino AcidReceptor in Rodent Brain. *Neurosci. Letters.* **113:** 78-83 (1990).

GLUTAMATE, NMDA, GLYCINE (STRYCHNINE-INSENSITIVE) SITE
BINDING ASSAY

Reference Compounds	Ki (nM)
■ MDL 105,519	17.1
▫ Glycine	181.3
● 5,7-DCKA	203.1
× D-Serine	389.7
◆ HA 966	1281.0

Assay Characteristics:

K_0 (binding affinity):	50 nM
B_{max} (receptor number):	29 pmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat cortical (with hippocampus) membranes
Radioligand:	[^{3}H]MDL-105,519 (50-90 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	MDL-105,519 - [0.3 µM]
Reference Compound:	MDL-105,519
Positive Control:	MDL-105,519
Incubation Conditions:	Reactions are carried out in 50 mM Tris-Acetate (pH 7.4) at room temperature for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glycine binding site.

Literature Reference:

Baron, et. al. Pharmacological characterization of MDL-105,519, an NMDA receptor glycine site antagonist. *European Journal of Pharmacology.* **323**: 181-192 (1997).

GLUTAMATE, NMDA AGONIST SITE
BINDING ASSAY
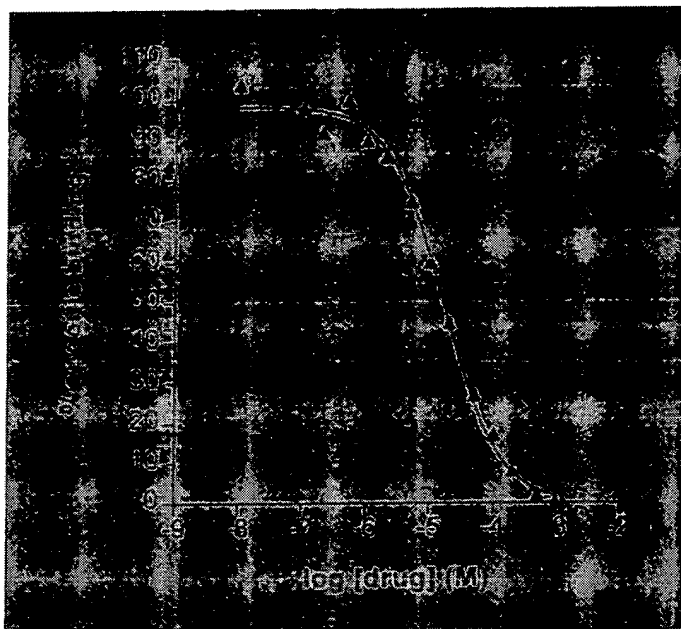
Reference Compounds	Ki (nM)
▲ NMDA	9,300

Assay Characteristics:

K_D (binding affinity):	7.0 nM
B_{max} (receptor number):	0.77 pmol/mg tissue

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]CGP 39653 (25-60 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	NMDA - [1.0 mM]
Reference Compound:	NMDA
Positive Control:	NMDA
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-Acetate (pH 7.4) at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtratio onto glass fiber filters. Radioactivity trapped onto the filter is determined and compared to control values in order to ascertain any interactions of test compound with the NMDA binding sites.

Literature Reference:

Lehmann, J., Hutchinson, A.J., et al. CGS 19755, A Selective and Competitive N-Methyl-D-Aspartate Type Excitatory Amino Acid Receptor Antagonist. *Jrnl. Pharmac. Exp. Ther.* **246**: 65-75 (1988) with modifications.

Murphy, D.E., Schneider, J., et al. Binding of [^{3}H]-3-(2-Carboxypiperazin-4-yl)Propyl-1-Phosphonic Acid to Rat Brain Membranes: A Selective, High Affinity Ligand for N-Methyl-D-Aspartate Receptors. *Jrnl. Pharmac. Exp. Ther.* **240**: 778-784 (1987) with modifications.

GLUTAMATE, NMDA, PHENCYCLIDINE SITE
BINDING ASSAY
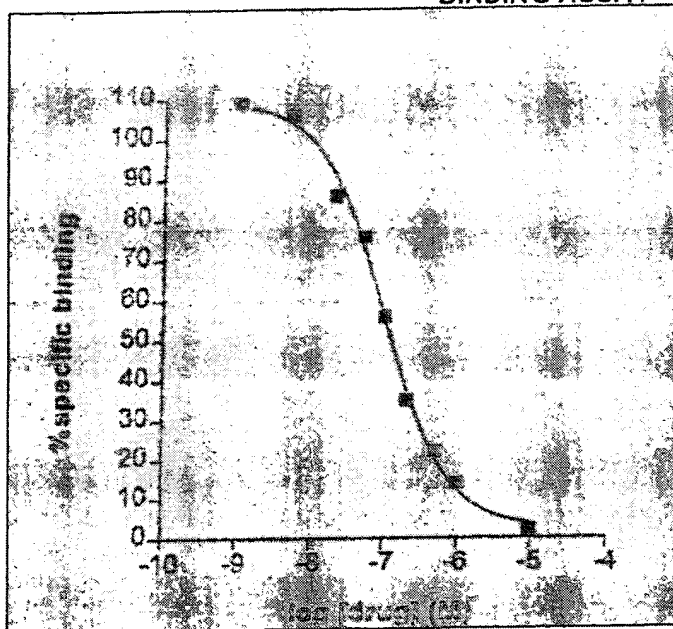
Reference Compounds	Ki (nM)
☐ (+) MK-801	7.9
PCP	77.0
Ketamine	994.5
(+)-3-PPP	>10,000
Haloperidol	>10,000

Assay Characteristics:

K_D (binding affinity):   23.5 nM
B_{max} (receptor number):   980 fmol/mg protein

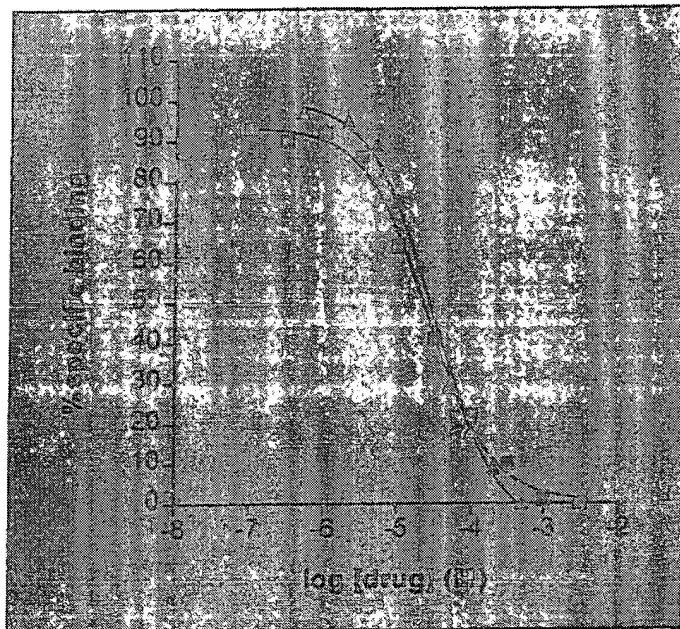## Materials and Methods:

Receptor Source: Rat forebrain membranes
Radioligand: [³H]TCP (40-60 Ci/mmol)
                          Final ligand concentration - [10.0 nM]
Non-specific Determinant: (+)MK-801 - [10 µM]
Reference Compound: (+)MK-801
Positive Control: (+)MK-801
Incubation Conditions: Reactions are carried out in 5.0 mM TRIS-HCl (pH 7.7) at 4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the phencyclidine binding site.

Literature Reference: Vignon, J., et al. [³H]TCP: A New Tool with High Affinity to PCP Receptors in Rat Brain. *Brain Research.* **280:** 194-196 (1983) with modifications.

Johnson, K. M., Sacaan, A. I., and Snell, L.D. Equilibrium Analysis of [³H]TCP Binding: Effects of Glycine, Magnesium, and NMDA Agonists. *Eur. Jml. Pharmacol.* **152:** 141-146 (1988).

Hampton, R.Y. et al. Stereospecific Binding of [³H]PCP in Brain Membranes. *Life Sciences.* **30:** 2147-2154 (1982).

GLUTAMATE, NMDA, MK-801 SITE
BINDING ASSAY
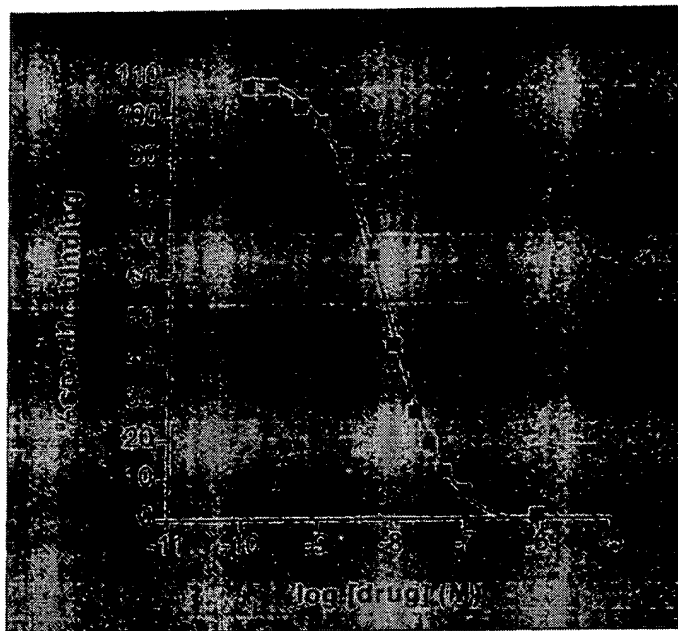
Reference Compounds	Ki (nM)
■ (+) MK-801	2.8
PCP	46.0
L-Glutamate	>100.0
NMDA	>100.0

Assay Characteristics:

K_D (binding affinity)	6.9 nM
B_{max} (receptor number)	830 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]MK-801 (15-30 Ci/mmol)
	Final Ligand Concentration - [2.6 nM]
Non-specific Determinant:	(+) MK-801 - [1.0 µM]
Reference Compound:	(+) MK-801
Positive Control:	(+) MK-801
Incubation Conditions:	Reactions are carried out in 20 mM HEPES (pH 7.5) at 25°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the (+)MK-801 binding site.

Literature Reference:

Javitt, D.C. and Zukin, S.R. Bioexponential Kinetics of [^{3}H]MK801 Binding: Evidence for Access to Closed and Open N-methyl-D-Aspartate Receptor Channels. *Mol. Pharmac.* **35:** 387 (1989) with modifications.

Foster, A.C. and Wong, E.H.F. The Novel Anticonvulsant MK-801 Binding to the Activated State of the NMDA Receptor in Rat Brain. *Brit. Jrnl. Pharmac.* **91:** 403-409 (1987).

GLYCINE, STRYCHNINE-SENSITIVE
BINDING ASSAY
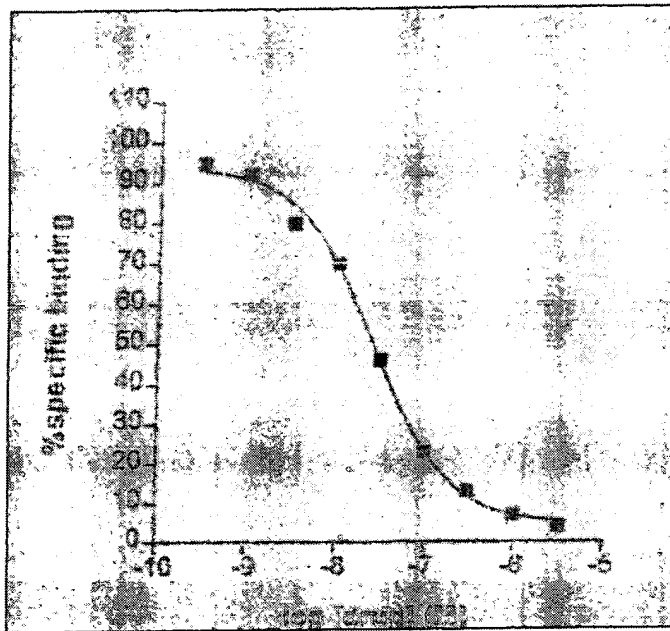
Reference Compounds	Ki (nM)
▢ Strychnine nitrate	70.0
Glycine	>10,000
β-Alanine	>100,000

Assay Characteristics:

K_D (binding affinity):	28.0 nM
B_{max} (receptor number):	3.8 pmol/mg protein

Materials and Methods:

Receptor Source:	Rat spinal cord membranes
Radioligand:	[³H]Strychnine (15-40 Ci/mmol)
	Final ligand concentration - [16.0 nM]
Non-specific Determinant:	Strychnine nitrate - [1.0 mM]
Reference Compound:	Strychnine nitrate
Positive Control:	Strychnine nitrate
Incubation Conditions:	Reactions are carried out in 50 mM Na-KPO₄ (pH 7.1) containing 200 mM NaCl at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity is determined and compared to control values in order to ascertain any interactions of test compound with the glycine (strychnine) sensitive binding site.

Literature Reference:

Young, A.B.,and Snyder, S.H. Strychnine Binding in Rat Spinal Cord Membranes Associated with the Synaptic Glycine Receptors: Cooperativity of Glycine Interactions. *Mol. Pharmacol.* **10:** 790-809 (1974) with modifications.

Ruiz-Gomez, A., et al. Thermodynamics of Agonist and Antagonist Interaction with the Strychnine Sensitive Glycine Receptor. *Jrnl. Neurochem.* **52(6):** 1775-1785 (1988).

GLUTAMATE TRANSPORT
BINDING ASSAY
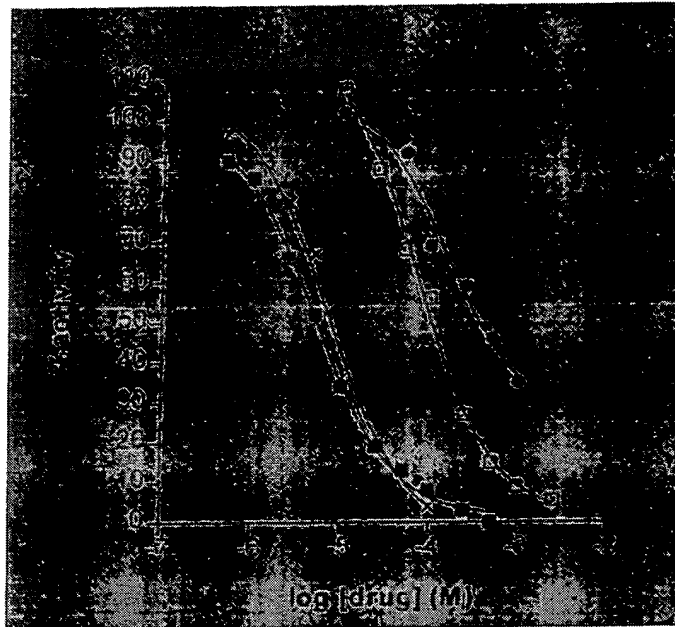
Reference Compounds	IC50 (uM)
■ D-Aspartate	6.0
× L-Glutamate	9.0
□ L-α-Amino adipate	69.5
● Kainate (partial inhibition)	96.0

Assay Characteristics:

Materials and Methods:

Receptor Source:	Rat cerebellar synaptosomes
Radioligand:	[³H]Glutamate (40-80 Ci/mmol)
	Final ligand concentration - [90 nM]
Non-specific Determinant:	D-Aspartate - [1.0 mM]
Reference Compound:	D-Aspartate
Positive Control:	D-Aspartate
Incubation Conditions:	Reactions are carried out in Krebs/HEPES buffer (pH 7.4) at 30°C for 3 minutes. The reaction is terminated by addition of ice cold buffer followed by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glutamate uptake site.

Literature Reference: Ferkany, J.W. and Coyle, J.T. Heterogeneity of Sodium-Dependent Excitatory Amino Acid Uptake Mechanisms in Rat Brain. *Jrnl. Neuroscience Res.* **16:** 491-503 (1986).

HISTAMINE, H$_2$
BINDING ASSAY
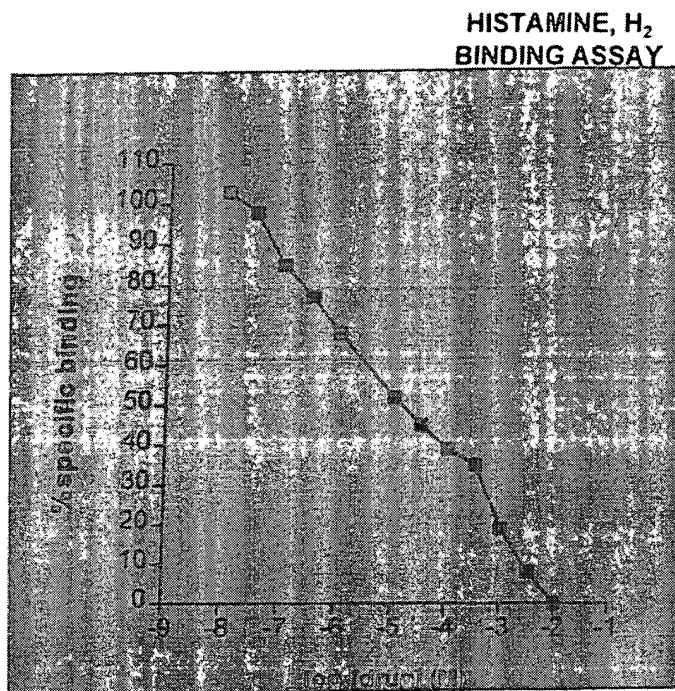
Reference Compounds   Ki (nM)
—□— Cimetidine   13,000

Assay Characteristics:

K_D (binding affinity):   9.4 μM
B_{max} (receptor number):   212 fmol/mg protein

Materials and Methods:

Receptor Resource: Guinea pig striatal membranes
Radioligand: [^{3}H]Tiotidine (70-90 Ci/mmol)
      Final ligand concentration - [4.0 nM]
Non-specific Determinant: Cimetidine - [10 mM]
Reference Compound: Cimetidine
Positive Control: Cimetidine
Incubation Conditions: Reactions are carried out in 50 mM Na-KPO$_4$ (pH 7.4) at 25°C for 20 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the histamine$_2$ binding site.

Literature Reference:

Gajtkowski, et al. Specific Binding of [^{3}H]Tiotidine to Histamine H$_2$ Receptors in Guinea Pig Cerebral Cortex. *Nature.* **304:** 65-67 (1983) with modifications.

Martinez-Mur, M.I., Pollard, H., Moreau, J., et al. Three Histamine Receptors (H$_1$, H$_2$, and H$_3$) Visualized in the Brain of Human and Non-Human Primates. *Brain Res.* **526:** 322-327 (1990).

Haaksma, E.E.J., Leurs, R. and Timmerman, H. Histamine Receptors: Subclasses and Specific Ligands. *Pharmac. Ther.* **47:** 73-104 (1990).

HISTAMINE, H₁
BINDING ASSAY
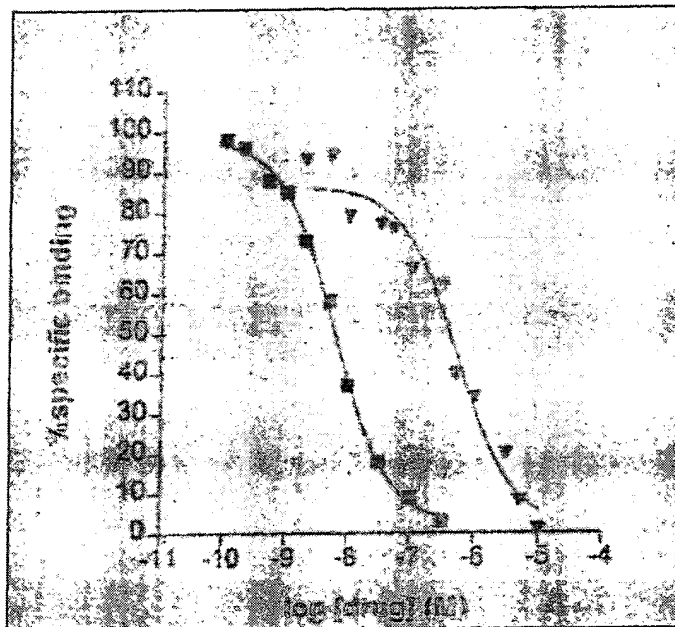
Reference Compounds		Ki (nM)
	Pyrilamine	1.9
☐	Triprolidine	3.3
	Cyproheptadine	8.5
▽	Chlorpheniramine	103.0
	Cimetidine	>10,000
	Dimaprit	>10,000

Assay Characteristics:

K_D (binding affinity): 1.3 nM
B_{max} (receptor number): 6.2 fmol/mg tissue (wet weight)
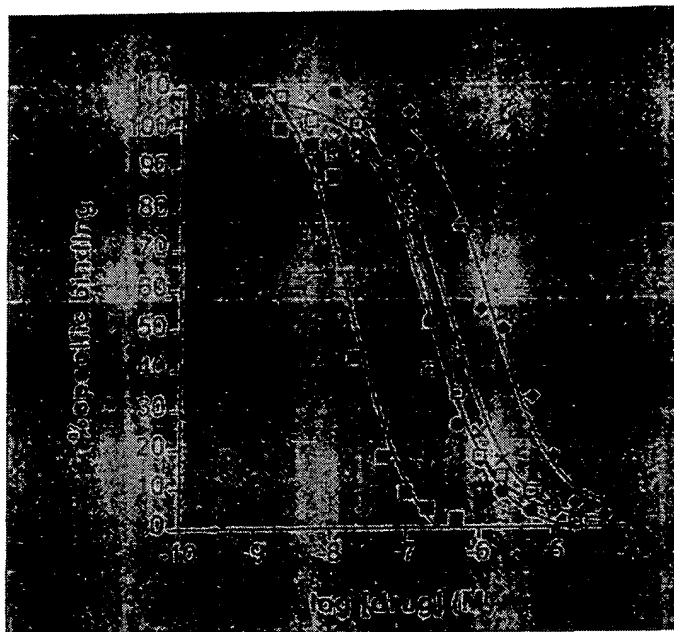
Materials and Methods:

Receptor Resource:	Bovine cerebellar membranes
Radioligand:	[³H]Pyrilamine (15-25 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	Triprolidine - [10 µM]
Reference Compound:	Triprolidine
Positive Control:	Triprolidine
Incubation Conditions:	Reactions are carried out in 50 mM Na-KPO₄ (pH 7.5) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the histamine₁ binding site.

Literature Reference:

Chang, et al. Heterogeneity of Histamine H₁-Receptors: Species Variations in [³H]Mepyramine Binding of Brain Membranes. *Journal of Neurochemistry.* **32:** 1653-1663 (1979) with modifications.

Martinez-Mir, M.I., Pollard, H., Moreau, J., et al. Three Histamine Receptors (H₁, H₂, and H₃) Visualized in the Brain of Human and Non-Human Primates. *Brain Res.* **526:** 322-327 (1990).

Haaksma, E.E.J., Leurs, R. and Timmerman, H. Histamine Receptors: Subclasses and Specific Ligands. *Pharmac. Ther.* **47:** 73-104 (1990).

IMIDAZOLINE₁ BINDING ASSAY
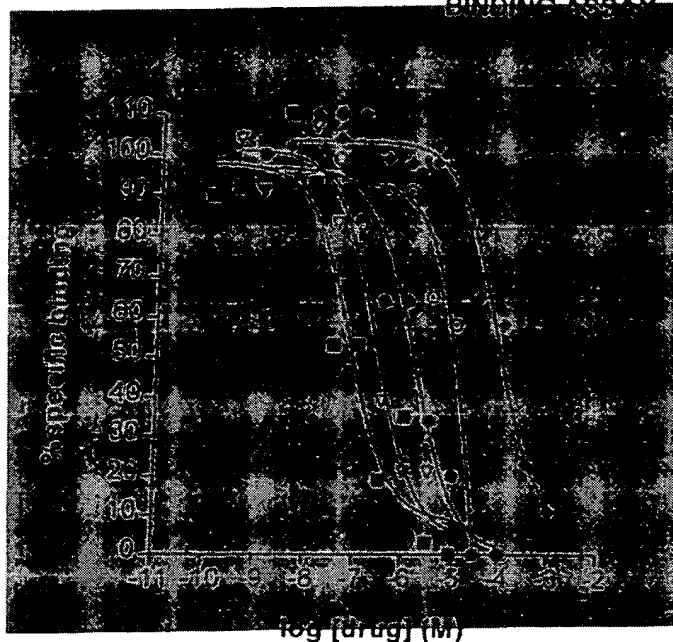
Reference Compounds	Ki (nM)
■ Iodo-clonidine	40.0
▽ Amino-clonidine	227.4
● Guanabenz	1,410
○ 2-BFI	25,740
◆ Idazoxan	71,070

Assay Characteristics:

K_D (binding affinity):	K_D = 30 nM
B_{max} (receptor number):	B_{max} = 50 fmol/mg protein

Materials and Methods:

Receptor Source:	PC12 cell membranes
Radioligand:	[^{125}I]-Iodoclonidine (1100Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	Iodoclonidine - [10.0 μM]
Reference Compound:	Iodoclonidine
Positive Control:	Iodoclonidine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM EDTA, 5 mM EGTA, 5 mM $MgCl_2$ and 30 μM norepinephrine at room temperature for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the imidazoline₁ binding site.

Literature Reference:	Steffen, G., Dendorfer, A., and Dominiak, P. Imidazoline binding sites on PC12 cells and bovine chromaffin cells. *Ann. N.Y. Acad. Sci.* **763**: 157-162 (1995), with modifications.
	Piletz, J.E., Zhu, H.E., and Chikkala, D.N., Comparison of Ligand Binding Affinities at Human I₁-Imidazoline Binding Sites and the High Affinity State of Alpha-2 Adrenergic Subtypes. *JPET.* **279**: 694-7002 (1996).

HISTAMINE, H₃
BINDING ASSAY
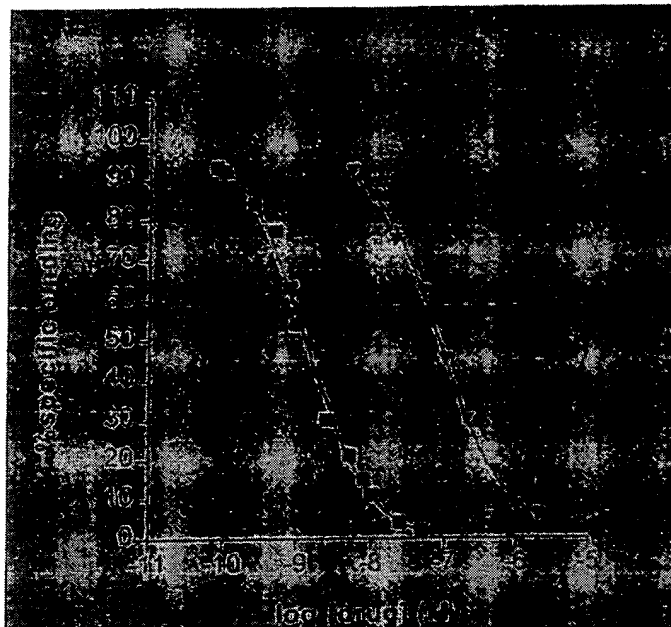
Reference Compounds	Ki (nM)
■ R(-)-α-Methylhistamine	0.79
▼ Histamine	59.3

Assay Characteristics:

K_D (binding affinity):	0.37 nM
B_{max} (receptor number):	73 fmol/mg protein

Materials and Methods:

Receptor Resource:	Rat forebrain membranes
Radioligand:	[³H]Nª-methylhistamine (80 - 90 Ci/mmol)
	Final ligand concentration - [0.2 nM]
Non-specific Determinant:	R(-)-α-methylhistamine - [0.1 nM]
Reference Compound:	R(-)-α-methylhistamine
Positive Control:	R(-)-α-methylhistamine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) for 60 minutes at 30°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the histamine₃ binding site.

Literature Reference:

West, Robert E., et al. Identification of Two H₃-Histamine Receptor Subtypes. *Mol. Pharmacol.* **38**: 610-613 (1990) with modifications.

Arrang, J. M., Garbarg, M., Lancelot, J.C., et al. Highly Potent and Selective Ligands for Histamine H₃ Receptors. *Nature.* **327**: 117-123 (1987) with modifications.

INOSITOL TRIPHOSPHATE, IP$_3$
BINDING ASSAY
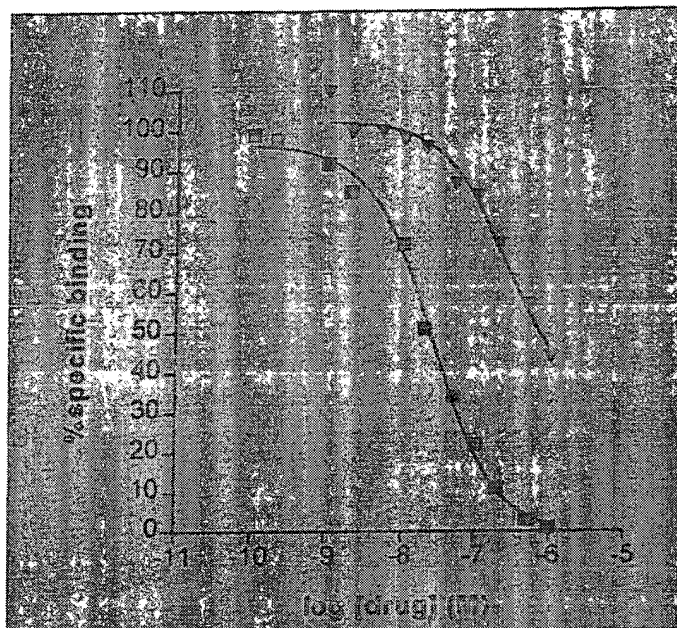
Reference Compounds	Ki (nM)
□ IP$_3$	12
▼ IP$_4$	672
IP$_5$	6,850
IP$_2$	>10,000
IP$_1$	>100,000

Assay Characteristics:

K_D (binding affinity):	40.0 nM
B_{max} (receptor number):	23 pmol/mg protein

Materials and Methods:

|	
Receptor Source:	Rat cerebellar membranes
Radioligand:	[^{3}H]IP$_3$ (30 - 50 Ci/mmol)
	Final ligand concentration - [4.0 nM]
Non-specific Determinant:	D-myo-inositol 1,4,5-triphosphate - [1.0 M]
Reference Compound:	D-myo-inositol 1,4,5-triphosphate
Positive Control:	D-myo-inositol 1,4,5-triphosphate
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 8.3) containing 1 mM EDTA at 0°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the inositol triphosphate binding site.

Literature Reference:

Worley, P., Baraban, J., Supattapone, S., Wilson, V. and Snyder, S. H. Characterization of Inositol Triphosphate Receptor Binding in Brain. *Jrnl. Biochem.* **262(25)**: 12132-12136 (1987) with modifications.

Willcocks, A.L., Cooke, A.M., Potter, B.V.L., and Nahorski, S.R. Stereospecific Recognition Sites for [^{3}H]Inositol (1, 4, 5)-Triphosphate in Particulate Preparations of Rat Cerebellum. *Biochem. Biophsy. Res. Comm.* **147**: 1071-1078 (1987).

IMIDAZOLINE$_2$
BINDING ASSAY
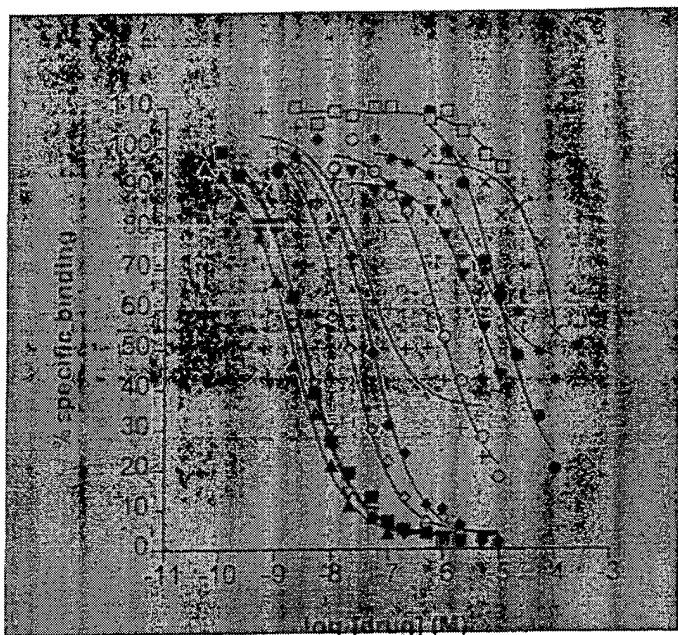
Reference Compounds	Ki (nM)
▲ BU 224**	0.15
▽ BU 239***	0.22
■ 2-BFI	0.24
◇ Idazoxane	1.20
◆ Guanabenz Acetate	2.60
+ Naphazoline	3.30
○ Tolazoline	57.90
▼ UK 14,304	250.0
* Rilmenidine	277.0
● Guanthedine	658.5
✕ RX 821002	>10,000
□ Oxymetazoline	>10,000
Agmatine	>10,000
Deprenyl	>10,000
Ro 41-1049	>10,000
16-64491	>10,000
Phenylbiguanide	>10,000
Raulwoscine	>100,000

Assay Characteristics:

K$_D$ (binding affinity):

K_D (High Affinity Site) = 0.08 nM *
K_D (Low Affinity Site) = 3.3 nM

B$_{max}$ (receptor number):

B_{max} (High Affinity Site) = 130 fmol/mg protein
B_{max} (Low Affinity Site) = 460 fmol/mg protein

Materials and Methods:

Receptor Source: Rabbit brain membranes
Radioligand: [^{3}H]2-BFI (5,7-(n)-2-(2-Benzofuranyl)-2-imidozoline) (70-90 Ci/mmol)
Final ligand concentration - [1.0 nM]

Non-specific Determinant: 2-BFI (5,7-(n)-2-(2-Benzofuranyl)-2-imidozoline) - [1.0 µM]
Reference Compound: 2-BFI (5,7-(n)-2-(2-Benzofuranyl)-2-imidozoline)
Positive Control: 2-BFI (5,7-(n)-2-(2-Benzofuranyl)-2-imidozoline) .
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 1 mM MgCl$_2$ at 25°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the imidazoline$_2$ binding site.

Literature Reference: Brown, C.M., MacKinnon, A.C., McGrath, J.C., Spedding, M., and Kilpatrick, A.T. Alpha-2 Adrenoceptor Subtypes and Imidazoline-Like Binding Sites in the Rat Brain. *Brit. Jrnl. Pharmacol.* **99(4):** 803-809 (1990) with modifications.

* = Imidazoline$_2$ assay demonstrates high and low affinity sites. Ki determinations were established using the K$_D$ of the high affinity site.
** = BU 224: 2-(4,5-Dihydroimidaz-2-yl)-quinoline
*** = BU 239: 2-(4,5-Dihydroimidaz-2-yl)-quinoxaline

LEUKOTRIENE B₄, LTB₄
BINDING ASSAY
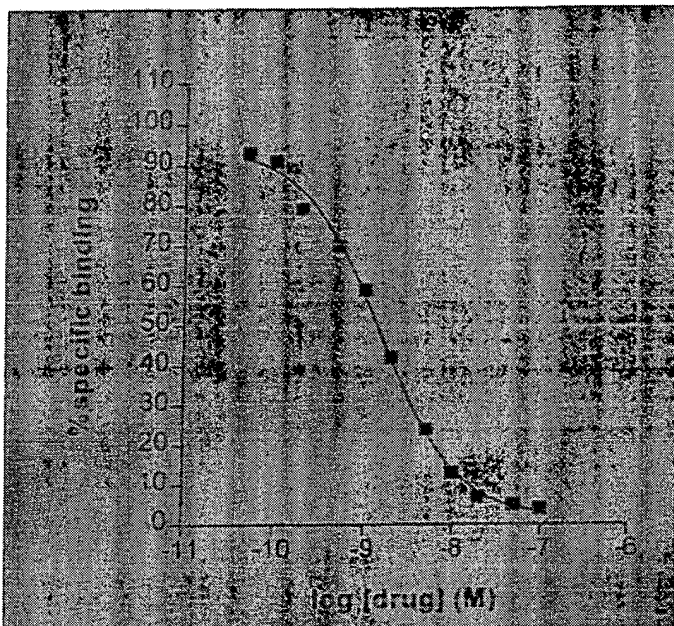
Reference Compounds	Ki (nM)
■ LTB₄	0.8
20-OH-LTB₄	2.9
LTD₄	2,640
Thromboxane	>10,000

Assay Characteristics:

K_d (binding affinity):	1.0 nM
B_{max} (receptor number):	250 fmol/mg protein

Materials and Methods:

Receptor Source:	Guinea pig spleen membranes
Radioligand:	[³H]Leukotriene B₄ (160-240 Ci/mmol)
	Final ligand concentration - [0.48 nM]
Non-specific Determinant:	Leukotriene B₄ - [300 nM]
Reference Compound:	Leukotriene B₄
Positive Control:	Leukotriene B₄
Incubation Conditions:	Reactions are carried out in a phosphate buffer (pH 7.4) containing NaCl, MgCl₂, EDTA, and bacitracin at 0-4°C for 2 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the LTB₄ binding site.

Literature Reference:

Cheng, Y., et al. [³H]LTB₄ Binding to Guinea Pig Spleen Membrane Preparation: A Rich Tissue Source for a High-Affinity Leukotriene B₄ Receptor Site. *Jrnl. Pharmac. Exp. Therapeut.* (1986) with modifications.

Gardiner, P.J., Abram, T.S., and Cuthbert, N.J. Evidence for Two Leukotriene Receptor Types in the Guinea Pig Isolated Ileum. *Eur. Jrnl. Pharmac.* **182:** 291-299 (1990).

INTERLEUKIN-1-ALPHA, $IL_1\alpha$ (HUMAN RECOMBINANT)
BINDING ASSAY
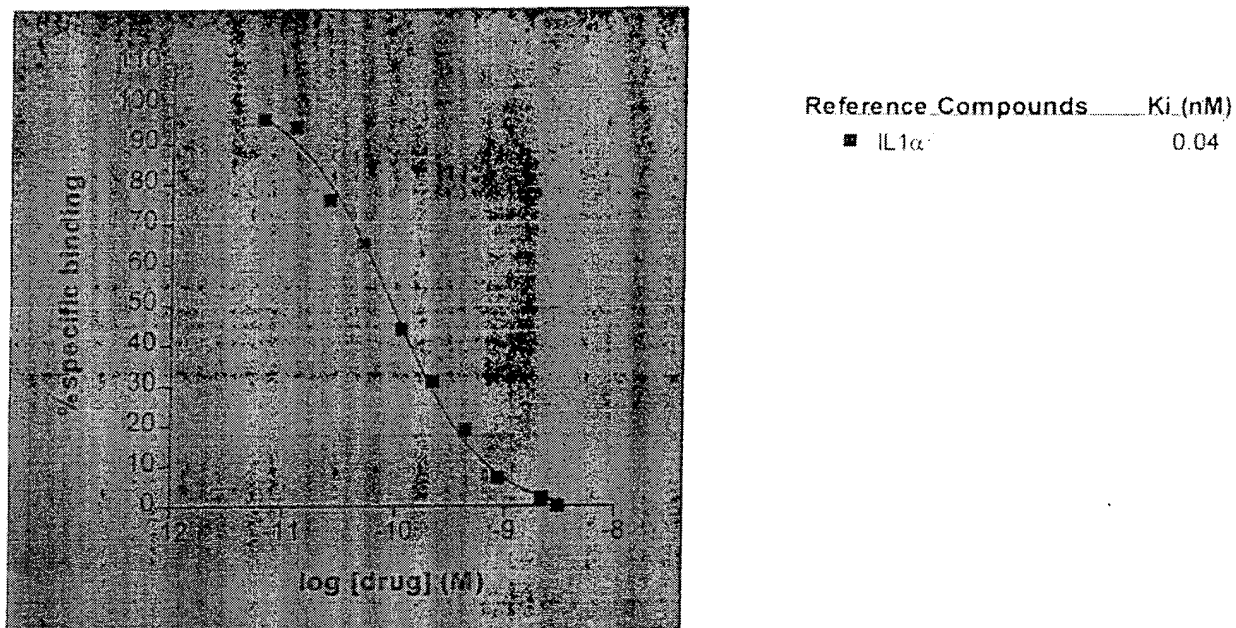
Assay Characteristics:

K_D (binding affinity):	0.08 nM
B_{max} (receptor number):	15 pM
Degree of Specific Binding:	65%(Non-specific binding determined using 4 nM of interleukin-1α, human)

Materials and Methods:

|	
Receptor Source:	$IL1\alpha$ Human recombinant expressed in CHO-IL-1R cells
Radioligand:	[^{125}I]Interleukin-1α (2200 Ci/mmol)
Reference Compound:	Interleukin-1α, human
Positive Control:	Interleukin-1α, human
Incubation Conditions:	Cells are resuspended to a concentration of 1.25×10^6/ml. Reactions are carried out in PBS (pH 7.4) containing BSA and bacitracin at 37°C for 3 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned $IL1\alpha$ binding site.

Literature Reference:

Kobayashi, Y., Oppenheim, J.J., and Matsushima, K. Calcium Dependent Binding of Phosphorylated Human Pre-Interleukin-1α to Phospholipids. *Jrnl. Biochem.* **107:** 666-670 (1990) with modifications.

Chin, J. E. and Horuk, R. Interleukin 1 Receptors on Rabbit Articular Chondrocytes: Relationship Between Biological Activity and Receptor Binding Kinetics. *The FASEB Jrnl.* **4:** 1481-1487 (1990) with modifications.

MELATONIN
BINDING ASSAY
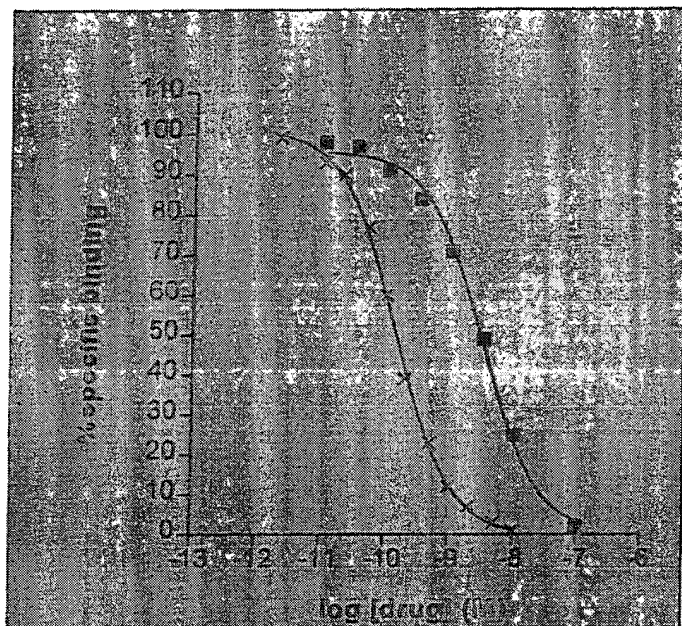
Reference Compounds	Ki (nM)
× 2-Iodomelatonin	0.07
▫ Melatonin	1.00
6-Hydroxymelatonin	10.00
N-Acetyl5HT	1,000
5-Methyoxytryptol	7,300
5-Methoxytryptamine	48,500
5-Methoxyindole	97,000
6-Methoxytryptamine	>100,000

Assay Characteristics:

K_d (binding affinity):	66 pM
B_{max} (receptor number):	0.48 fmol/mg tissue (wet weight)

Materials and Methods:

|	
Receptor Source:	Chicken brain membranes
Radioligand:	[^{125}I]-2-Iodomelatonin
	Final ligand concentration - [70 pM]
Non-specific Determinant:	Melatonin - [1.0 µM]
Reference Compound:	Melatonin
Positive Control:	Melatonin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.5) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the Melatonin binding site.

Literature Reference:

Dubocovich, M. L., et al. 2-[^{125}I]Iodomelatonin Labels Sites with Identical Pharmacological Characteristics in Chicken Brain and Chicken Retina. *Eur. Jrnl. Pharm.* **162:** 289-199 (1989) with modifications.

Pontoire, C., Bernard, C., et al. Characterization of Melatonin Binding Sites in Chicken and Human Intestines. *Eur. Jrnl. Pharmac.* **247(2):** 111-118 (1993).

LEUKOTRIENE D$_4$, LTD$_4$
BINDING ASSAY
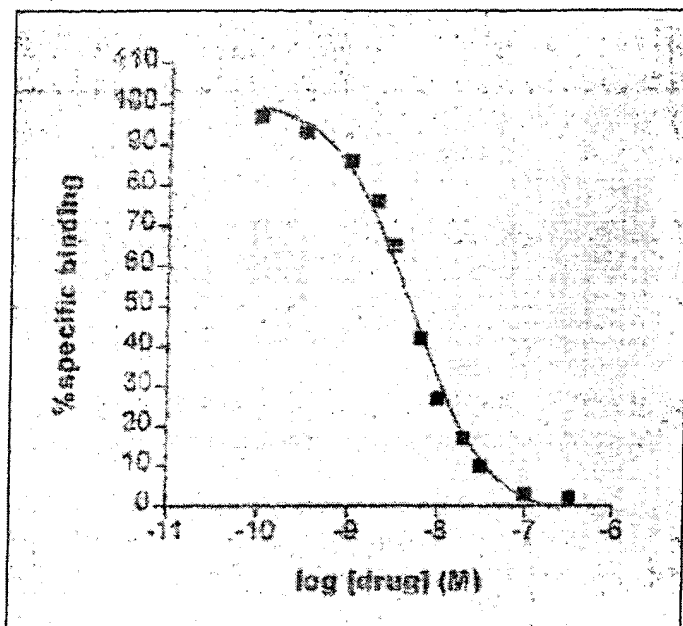
Reference Compounds	Ki (nM)
▫ Leukotriene D$_4$	2.4
20-OH-LTD$_4$	>1,000
Thromboxane	>10,000

Assay Characteristics:

K$_d$ (binding affinity):   5.0 nM

B$_{max}$ (receptor nimber):   182 fmol/mg protein

Materials and Methods:

Receptor Source: Guinea pig lung membranes

Radioligand: [^{3}H]Leukotriene D$_4$ (100-240 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific Determinant: Leukotriene D$_4$ - [1.0 uM]

Reference Compound: Leukotriene D$_4$

Positive Control: Leukotriene D$_4$

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the leukotriene D$_4$ binding site.

Literature Reference: Norman, P., Abram, T.S., Cuthbert, N.J., and Gardiner, P.J. The Inhibition of [^{3}H]Leukotriene D$_4$ Binding to Guinea Pig Lung Membranes. The Correlation of Binding Affinity with Activity on the Guinea Pig Ileum. *Eur. Jrnl. Pharmac.* **182**: 301-312 (1990) with modifications.

Hogaboom, et al. Peptidoleukotrienes: Distinct Receptors for Leukotriene C$_4$ and D$_4$ in the Guinea Pig Lung. *Biochem. & Biophys. Res. Communic.* **116**: 1136-1143 (1983).

MUSCARINIC, NON-SELECTIVE, PERIPHERAL
BINDING ASSAY
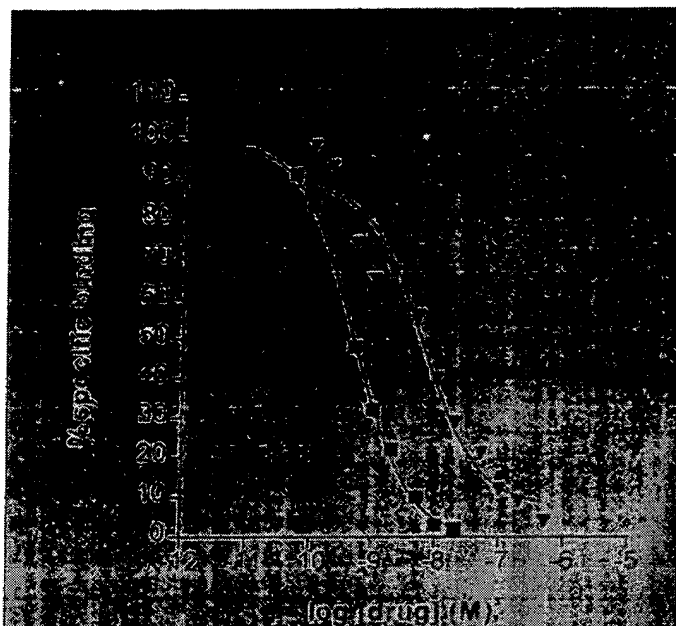
Reference Compounds	Ki (nM)
■ Atropine	0.2
Scopolamine	1.0
Dexetimide	1.1
▼ 4-DAMP Methiodide	2.6

Assay Characteristics:

K_D (binding affinity):	0.3 nM
B_{max} (receptor number):	5.0 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Guinea pig bladder membranes
Radioligand:	[^{3}H]Quinuclidinyl benzilate (QNB) (30-60 Ci/mmol)
	Final ligand concentration - [0.3 nM]
Non-specific Determinant:	Atropine - [1.0 uM]
Reference Compound:	Atropine
Positive Control:	Atropine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the muscarinic binding site.

Literature Reference:

Luthin, G. R. and Wolfe, B. B. Comparison of [^{3}H]Pirenzepine and [^{3}H]QNB Binding to Muscarinic Cholinergic Receptors in Rat Brain. *Jrnl. Pharmac. Exp. Ther.* **228**: 648-655 (1984) with modifications.

MUSCARINIC, NON-SELECTIVE, CENTRAL
BINDING ASSAY
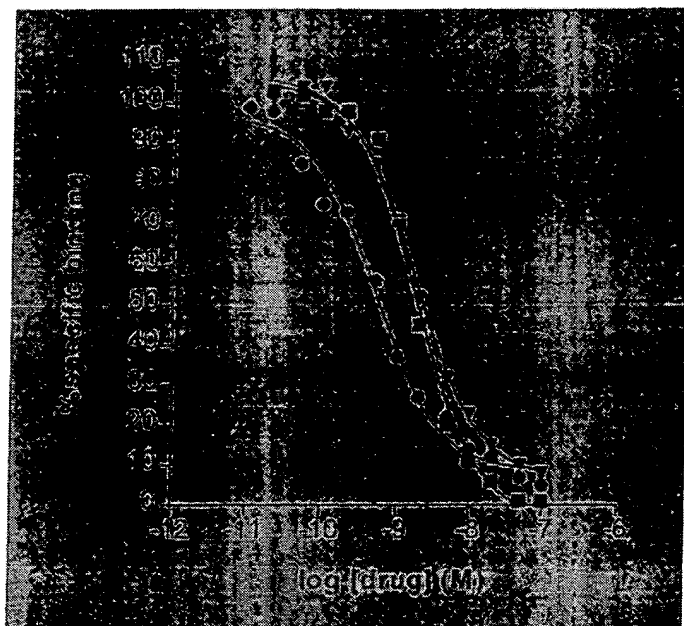
Reference Compounds	Ki (nM)
QNB	0.1
■ Atropine	0.1
● 4-DAMP Methiodide	0.3
▼ Scopolamine	0.3
Pirenzepine	40.0

Assay Characteristics:

K_D (binding affinity):	0.1 nM
B_{max} (receptor number):	17.0 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]Quinuclidinylbenzilate (QNB) (30-60 Ci/mmol)
	Final ligand concentration - [0.15 nM]
Non-specific Determinant:	Atropine - [0.1 uM]
Reference Compound:	Atropine
Positive Control:	Atropine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the muscarinic binding site.

Literature Reference:

Luthin, G.R. and Wolfe, B.B.   Comparison of [^{3}H]Pirenzepine and [^{3}H]Quinuclidinylbenzilate Binding to Muscarinic Cholinergic Receptors in Rat Brain. *Jrnl. Pharmac. Exp. Ther.* **228**: 648-655 (1984) with modifications.

Luthin, G.R. and Wolfe, B.B. [^{3}H]Pirenzepine and [^{3}H]QNB Binding to Brain Muscarinic Receptors. *Mol. Pharmac.* **26**: 164-169 (1984).

Yamamura, H.I. and Snyder, S.H.   Muscarinic Cholinergic Binding in Rat Brain. *Proc. Nat'l. Acad. Sci.* **71**: 1725-1729 (1974).

MUSCARINIC, M, (HUMAN RECOMBINANT)
BINDING ASSAY
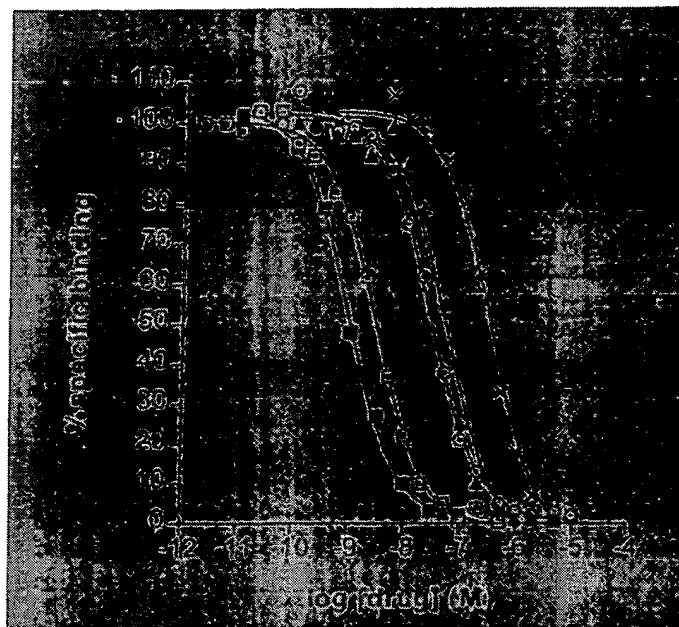
Reference Compounds	Ki (nM)
■ Scopolamine, MethylBr	0.09
□ 4-DAMP Methiodide	0.27
○ Pirenzepine	2.60
▲ HHSiD	5.00
× Methoctramine	29.70

Assay Characteristics:

K_D (binding affinity):	0.05 nM
B_{max} (receptor number):	4.2 pmole/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{3}H]-Scopolamine, N-Methyl Chloride (80-100 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specific Determinant:	Methylscopolamine bromide) - [1.0 μM]
Reference Compound:	(-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)
Positive Control:	(-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containin 10 mM MgCl$_2$, 1 mM EDTA for 60 minutes at 25°C. The reaction i terminated by rapid vacuum filtration onto glass fiber filters Radioactivity trapped onto the filters is determined and compared t control values in order to ascertain any interactions of tes compound(s) with the cloned muscarinic - M$_1$ binding site.

Literature Reference:

Buckley, N.J., Bonner, T.I., Buckley, C.M., and Brann, M.R Antagonist Binding Properties of Five Cloned Muscarini Receptors Expressed in CHO-K1 Cells. *Mol. Pharmacol.* **35**: 469 476 (1989) with modifications.

MUSCARINIC, M₁
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Atropine	0.4
Pirenzepine	4.5
Telenzepine	64.5

Assay Characteristics:

K_d (binding affinity):	2.2 nM
B_{max} (receptor number):	1.4 pmol/mg protein

Materials and Methods:

Receptor Source:	Bovine striatal membranes
Radioligand:	[³H]Pirenzepine (70-87 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Atropine - [0.1 μM]
Reference Compound:	Atropine
Positive Control:	Atropine
Incubation Conditions:	Reactions are carried out in 25 mM HEPES (pH 7.4) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the muscarinic₁ binding site.

Literature Reference:

Watson, M., Yamamura, H.I., and Roeske, W. A Unique Regulatory Profile and Regional Distribution of [³H]Pirenzepine Binding in the Rat Provide Evidence for Distinct M₁ and M₂ Muscarinic Receptor Subtypes. *Life Sciences.* **32:** 3001-3011 (1983) with modifications.

Luthin, G.R. and Wolfe, B.B. [³H]Pirenzepine and [³H]QNB Binding to Brain Muscarinic Cholinergic Receptors. *Molec. Pharmac.* **26:** 164-169 (1984).

MUSCARINIC, M$_2$ (HUMAN RECOMBINANT)
BINDING ASSAY
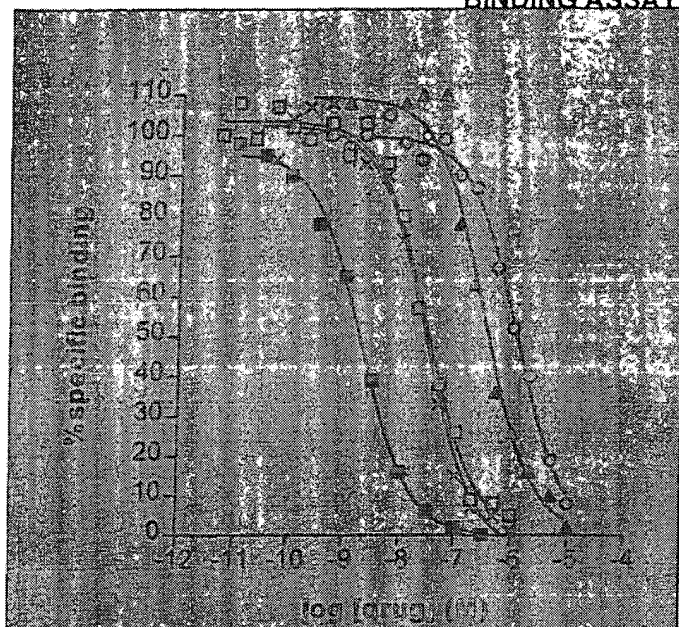
Reference Compounds	Ki (nM)
□ Scopolamine, MethylBr	0.3
□ 4-DAMP Methiodide	20.7
× Methoctramine	20.4
▲ HHSiD	212.7
○ Pirenzepine	832.9

Assay Characteristics:

K$_D$ (binding affinity):	0.2 nM
B$_{max}$ (receptor number):	2.1 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{3}H]-Scopolamine, N-Methyl Chloride (80-100 Ci/mmol) Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Methylscopolamine bromide - [1.0 μM]
Reference Compound:	(-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)
Positive Control:	(-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl$_2$, 1 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned muscarinic - M$_2$ binding site.

Literature Reference:	Buckley, N.J., Bonner, T.I., Buckley, C.M., and Brann, M.R Antagonist Binding Properties of Five Cloned Muscarinic Receptor Expressed in CHO-K1 Cells. *Mol. Pharmacol.* **35**: 469-476 (1989 with modifications.

MUSCARINIC, M$_2$
BINDING ASSAY
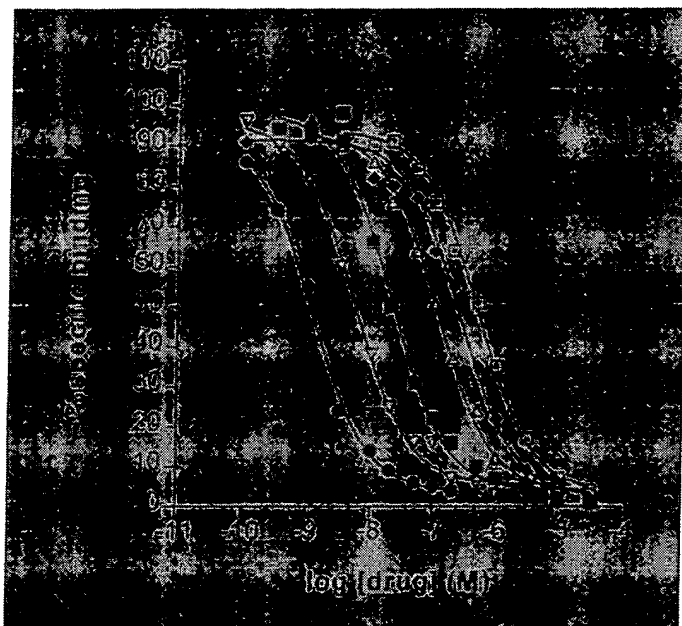
Reference Compounds		Ki (nM)
●	Atropine	0.7
▽	4-DAMP Methiodide	3.0
■	Methoctramine	11.8
△	AF-DX 116	63.0
◆	HHSID	151.7
□	Pirenzepine	273.5

Assay Characteristics:

K$_d$ (binding affinity):	6.4 nM
B$_{max}$ (receptor number):	2.1 pmol/mg protein

Materials and Methods:

Receptor Source:	Rat cardiac membranes
Radioligand:	[^{3}H]AF-DX 384 (70-120 Ci/mmol)
	Final ligand concentration - [3.0 nM]
Non-specific Determinant:	Methoctramine - [100 μM]
Reference Compound:	Methoctramine
Positive Control:	Methoctramine
Incubation Conditions:	Reactions are carried out in 10 mM Na-KPO$_4$ (pH 7.4) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the muscarinic$_2$ binding site.

Literature Reference:

Hammer, R., Giraldo, E. et al. Binding Profile of a Novel Cardioselective Muscarine Receptor Antagonist, AF-DX 116, to Membranes of Peripheral Tissues and Brain in the Rat. *Life Sciences.* **38:** 1653-1662 (1986) with modifications.

Wang, J.X., Roeske, W.R. et al. [^{3}H]AF-DX 116 Labels Subsets of Muscarinic Cholinergic Receptors in Rat Brain and Heart. *Life Sciences.* **41:** 1751-1760 (1987).

Elberlein, W.G., et al. Supplement: Subtypes Muscarinic Receptors IV. *TIPS.* **50** (1989).

MUSCARINIC, M₃ (HUMAN RECOMBINANT)
BINDING ASSAY
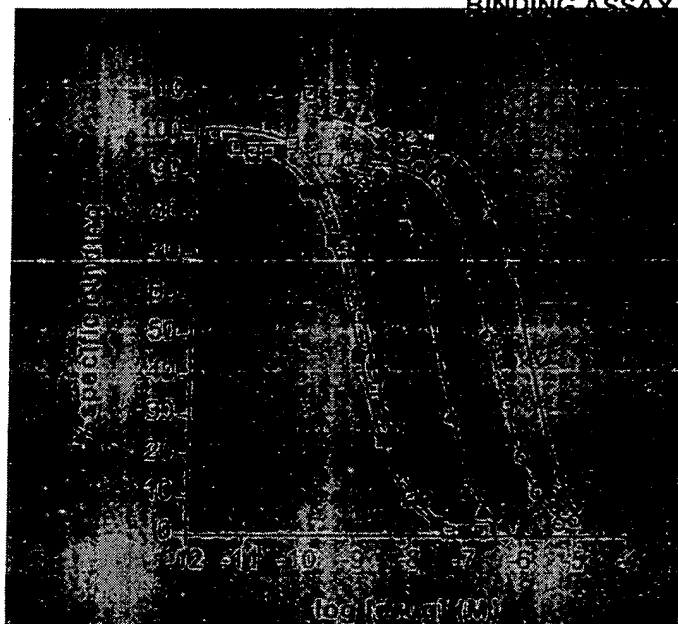
Reference Compounds	Ki (nM)
■ Scopolamine, MethylBr	0.3
□ 4-DAMP Methiodide	0.8
▲ HHSiD	14.5
○ Pirenzepine	153.3
× Methoctramine	700.0

Assay Characteristics:

K_D (binding affinity):   0.14 nM

B_{max} (receptor number):   4.0 pmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO cells

Radioligand: [³H]-Scopolamine, N-Methyl Chloride (80-100 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific Determinant: Methylscopolamine bromide - [1.0 µM]

Reference Compound: (-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)

Positive Control: (-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl₂, 1 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned muscarinic - M₃ binding site.

Literature Reference:

Buckley, N.J., Bonner, T.I., Buckley, C.M., and Brann, M.R Antagonist Binding Properties of Five Cloned Muscarinic Receptor Expressed in CHO-K1 Cells. *Mol. Pharmacol.* **35**: 469-476 (1989 with modifications.

MUSCARINIC, M₃
BINDING ASSAY
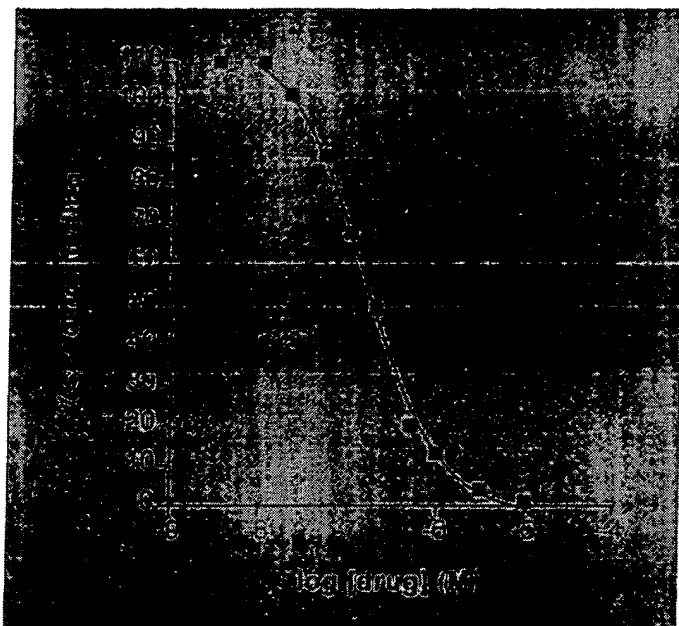
Reference Compounds	Ki (nM)
■ 4-DAMP Methiodide	37.5
HHSID	281.0

Assay Characteristics:

K_d (binding affinity):	1.4 nM
B_{max} (receptor number):	7.7fmol/mg protein

Materials and Methods:

Receptor Source: Guinea pig ileum membranes

Radioligand: [³H]-N-methylscopolamine (70-87 Ci/mmol)
Final ligand concentration - [1.0 nM]

Non-specific Determinant: Atropine - [10 uM]

Reference Compound: 4-DAMP methiodide

Positive Control: 4-DAMP methiodide

Incubation Conditions: Reactions are carried out in 30 mM HEPES (pH 7.4) containing 142 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl₂, 3.6 mM Na₂CO₃, 1 mM MgCl₂ and 5.6 mM glucose at 37°C for 2 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the muscarinic₃ binding site.

Literature Reference: Hanack, C., and Pfeiffer, A. Upper Gastrointestinal Porcine Smooth Muscle Expresses M₂ and M₃ Receptors. *Digestion.* **45:** 196-201 (1990) with modifications.

Vanderheyden, P., Gies, J-P., et al. Human M₁, M₂, and M₃ Muscarinic Cholinergic Receptors: Binding Characteristics of Agonists and Antagonists. *Jrnl. Neurolog. Sci.* **97:** 67-80 (1990).

Smith, T.D., Annis, S.J., et al. N-[³H]Methylscopolamine Labeling of Non-M₁, Non-M₂ Muscarinic Receptor Binding Sites in Rat Brain. *Jrnl. Pharmacol. Exp. Ther.* **256(3):** 1173-1181 (1990).

MUSCARINIC, M$_5$ (HUMAN RECOMBINANT) BINDING ASSAY
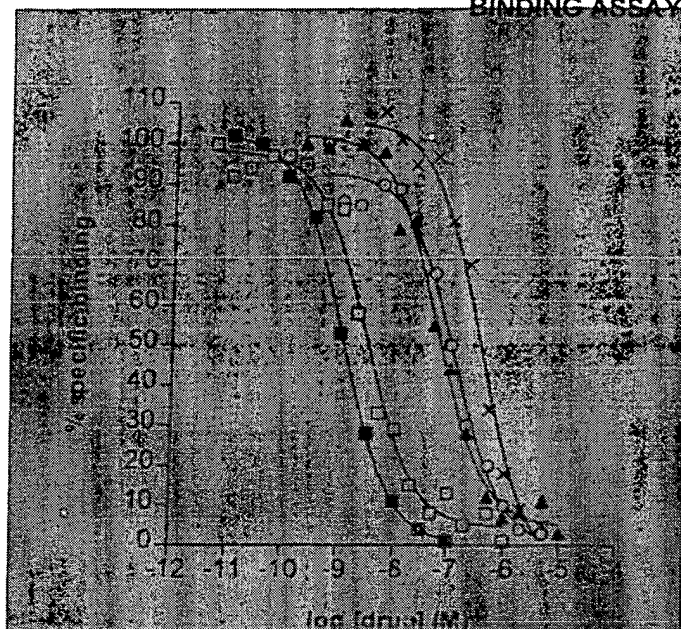
Reference Compounds	Ki (nM)
■ Scolopmine, MethylBr	0.5
▫ 4-DAMP Methiodide	2.4
▲ HHSiD	45.8
○ Pirenzepine	93.6
× Methoctramine	269.7

Assay Characteristics:

K$_D$ (binding affinity):   0.2 nM

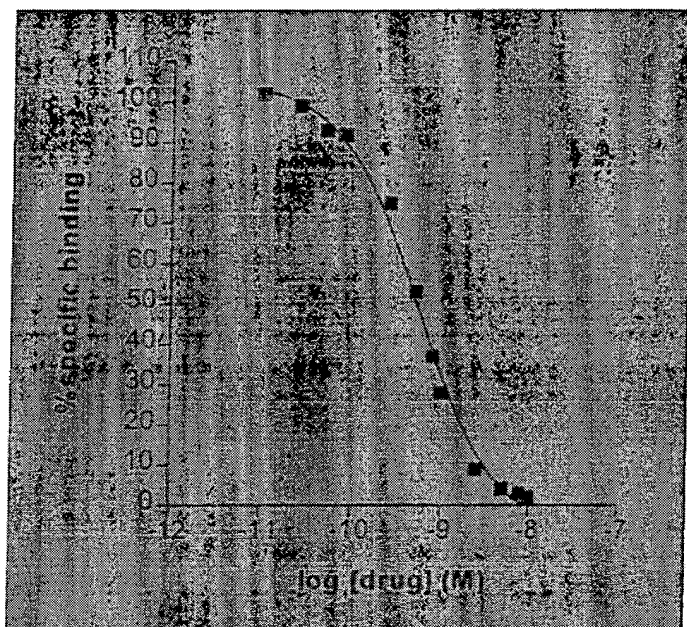B$_{max}$ (receptor number):   1.9 pmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO cells

Radioligand: [^{3}H]-Scopolamine, N-Methyl Chloride (80-100 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific Determinant: Methylscopolamine bromide - [1.0 µM]

Reference Compound: (-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)

Positive Control: (-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl$_2$, 1 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned muscarinic - M$_5$ binding site.

Literature Reference:

Buckley, N.J., Bonner, T.I., Buckley, C.M., and Brann, M.R Antagonist Binding Properties of Five Cloned Muscarinic Receptor Expressed in CHO-K1 Cells. *Mol. Pharmacol.* **35**: 469-476 (1989 with modifications.

MUSCARINIC, M$_4$ (HUMAN RECOMBINANT)
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Scopolamine, MethylBr	0.1
□ 4-DAMP Methiodide	3.3
▲ HHSiD	28.8
○ Pirenzepine	45.6
× Methoctramine	111.6

Assay Characteristics:

|	
K$_D$ (binding affinity):	0.09 nM
B$_{max}$ (receptor number):	4.3 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{3}H]-Scopolamine, N-Methyl Chloride (80-100 Ci/mmol)
	Final ligand concentration - [0.2 nM]
Non-specific Determinant:	Methylscopolamine bromide - [1.0 µM]
Reference Compound:	(-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)
Positive Control:	(-)-Scopolamine, Methyl-, bromide (Methylscopolamine bromide)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl$_2$, 1 mM EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned muscarinic - M$_4$ binding site.

Literature Reference:

Buckley, N.J., Bonner, T.I., Buckley, C.M., and Brann, M.R Antagonist Binding Properties of Five Cloned Muscarinic Receptor Expressed in CHO-K1 Cells. *Mol. Pharmacol.* **35**: 469-476 (1989 with modifications.

NEUROKININ, NK₁ (HUMAN RECOMBINANT) BINDING ASSAY
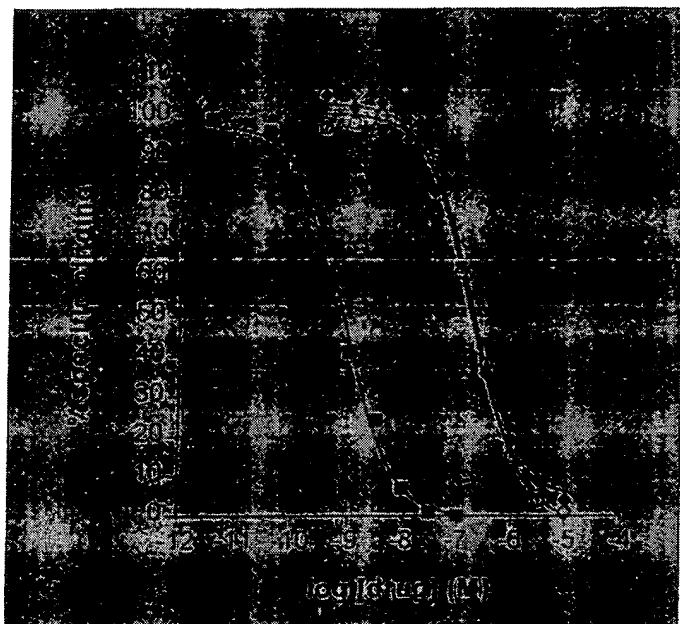
Reference Compounds	Ki (nM)
■ Substance P	0.3
◆ NKA	46.0
○ Eledoisin	63.0

Assay Characteristics:

K_D (binding affinity):	0.2 nM
B_{max} (receptor number):	1.4 pmol/mg protein

Materials & Methods:

Receptor Source:	Human Recombinant expressed in CHO cells
Radioligand:	[³H]Substance P (20-30 Ci/mmol)
	Final ligand concentration - [0.3 nM]
Non-specific Determinant:	Substance P - [0.1 uM]
Reference Compound:	Substance P
Positive Control:	Substance P
Incubation Conditions:	Reactions are carried out in 20 mM HEPES (pH 7.4) containing 0.01% BSA and 1 mM MnCl₂ for 1 hour at room temperature. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the neurokinin A binding site.

Literature Reference:

Bahouth, S.W. and Musacchio, J.M. Specific Binding of [³H]Substance P to the Rat Submaxillary Gland. *Jrnl. Pharm. & Exp. Ther.* **234**: 326-336 (1985) with modifications.

McLean, S., Ganong, A., Seymour, P.A., et al. Pharmacology of CP-99,994; a Nonpeptide Antagonist of the Tachykinin NK1 Receptor. *Jrnl. Pharm. & Exp. Ther.* **267**: 472-479 (1993).

Regoli, D. and Nantel, F. Pharmacology of Neurokinin Receptors. *Biopolymers.* **31**: 777-783 (1991).

NEUROKININ, NK₁
BINDING ASSAY
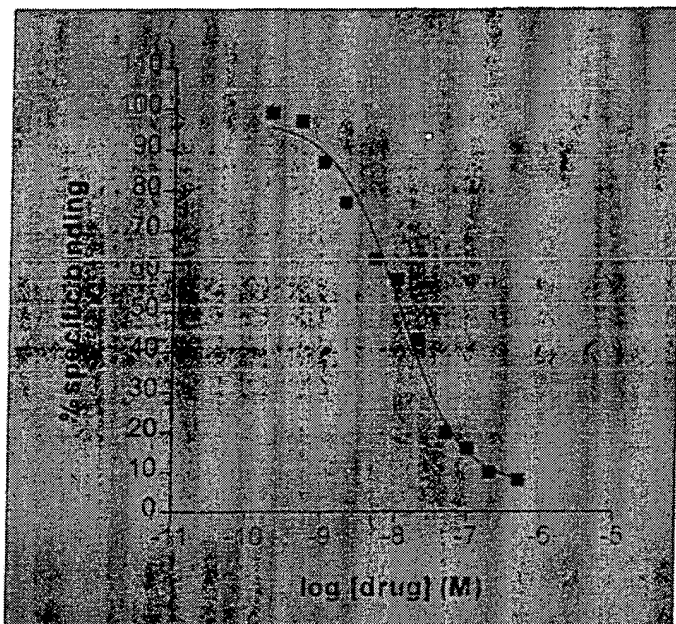
Reference Compounds	Ki (nM)
■ Substance P	6.0
Physalaemin	42.3
Eledoisin	91.9
Kassinin	295.0
Substance $P_{(4-11)}$	2,290.0

Assay Characteristics:

K_D (binding affinity): 1.4 nM
B_{max} (receptor number): 134.7 fmol/mg protein

Materials & Methods:

Receptor Source: Rat submaxillary gland membranes
Radioligand: [³H]Substance P (20-50 Ci/mmol)
Final ligand concentration - [1.4 nM]
Non-specific Determinant: Substance P - [1.0 uM]
Reference Compound: Substance P
Positive Control: Substance P
Incubation Conditions: Reactions are carried out in 20 mM HEPES (pH 7.4), 5 mM MgCl₂, 30 mM KCl, 0.02% BSA, 0.1 mM thiorphan for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the Substance P binding site.

Literature Reference: Bahouth, S.W. and Musacchio, J.M. Specific Binding of [³H]Substance P to the Rat Submaxillary Gland. *Jrnl. Pharm. & Exp. Ther.* **234**: 326-336 (1985) with modifications.

McLean, S., Ganong, A., Seymour, P.A., et al. Pharmacology of CP-99,994: a Nonpeptide Antagonist of the Tachykinin NK1 Receptor. *Jrnl. Pharm. & Exp. Ther.* **267**: 472-479 (1993).

Regoli, D. and Nantel, F. Pharmacology of Neurokinin Receptors. *Biopolymers.* **31**: 777-783 (1991).

NEUROKININ, NK₃ (NEUROKININ B) BINDING ASSAY

Reference Compound	Ki (nM)
Kassinin	4.7
Physalaemin	10.4
■ Eledoisin	13.4
NKA	27.1
NKB	77.0
Substance P	83.5

Assay Characteristics:

K_D (binding affinity):	1.5 nM
B_{max} (receptor number):	2.7 pmol/mg protein

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{125}I]Eledoisin (2200 Ci/mmol)
	Final ligand concentration - [0.1 nM]
Non-specific Determinant:	Eledoisin - [1.0 μM]
Reference Compound:	Eledoisin
Positive Control:	Eledoisin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 0.03% BSA, 60 μg/ml bacitracin, 6 μg/ml leupeptin, 6 μg/ml chymostatin, and 3 mM MnCl₂ for 2 hours at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the NK₃ binding site.

Literature Reference:

Mussap, C. J. and Burcher, E. [^{125}I]BH Scyliorhinin II: A Novel, Selective Radioligand for the NK₃ Receptor in Rat Brain. *Peptides.* **11**: 827-836 (1990) with modifications.

Regoli, D. and Nantel, F. Pharmacology of Neurokinin Receptors. *Biopolymers.* **31**: 777-783 (1991).

NEUROKININ, NK$_2$ (NK$_A$) (HUMAN RECOMBINANT) BINDING ASSAY
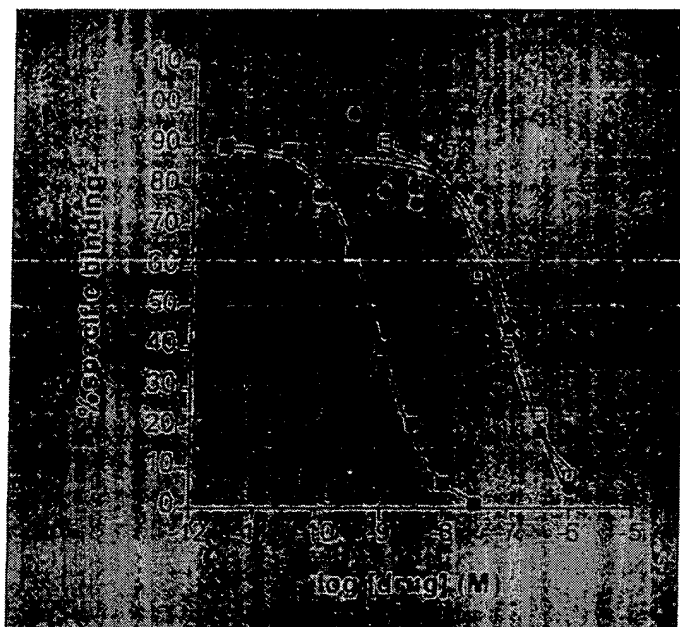
Reference Compounds	Ki (nM)
■ Neurokinin A	0.5
□ Eledoisin	63.9
● Substance P	128.4

Assay Characteristics:

K$_D$ (binding affinity):	0.5 nM
B$_{max}$ (receptor number):	5.0 pmol/mg protein

Materials & Methods:

|	
Receptor Source:	Human Recombinant expressed in CHO cells
Radioligand:	[^{125}I]Neurokinin A (2000 Ci/mmol)
	Final ligand concentration - [0.1 nM]
Non-specific Determinant:	Neurokinin A - [1.0 uM]
Reference Compound:	Neurokinin A
Positive Control:	Neurokinin A
Incubation Conditions:	Reactions are carried out in 20 mM HEPES (pH 7.4) containing 0.02% BSA and 1 mM MnCl$_2$ for 4 hours at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the neurokinin A binding site.

Literature Reference:

Burcher, E., Buck, S.H., Lovenberg, W. Characterization and Autoradiographic Localization of Multiple Tachykinin Binding Sites in Gastrointestinal Tract and Bladder. *Jrnl. Pharmac. & Exp. Ther.* **236 (3):** 819-831 (1986) with modifications.

Regoli, D. and Nantel, F. Pharmacology of Neurokinin Receptors. *Biopolymers.* **31:** 777-783 (1991).

NEUROPEPTIDE, NPY₁ (HUMAN)
BINDING ASSAY
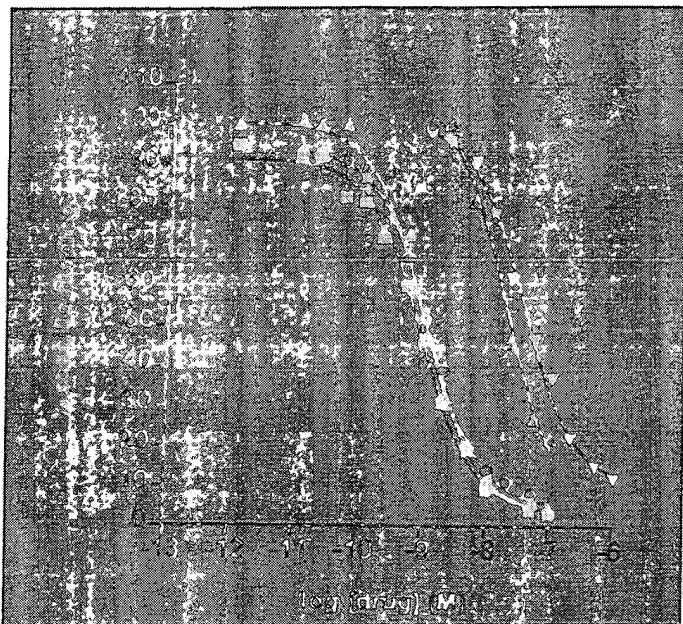
Reference Compounds	Ki (nM)
△ PYY (porcine)	0.8
○ [Leu31, Pro34]NPY (human)	1.2
▣ NPY (porcine)	2.5
△ NPY 18-36	25.8
▼ Pancreatic Polypeptide-1	50.1

Assay Characteristics:

K_D (binding affinity): 0.8 nM
B_{max} (receptor number): 700 fmol/mg protein

Materials and Methods:

Receptor Source: SK-N-MC Cells
Radioligand: [^{125}I]Peptide YY (PYY) (4000 Ci/mmol)
  Final ligand concentration - [0.025 nM]
Non-specific Determinant: NPY (porcine) - [1.0 µM]
Reference Compound: NPY (porcine)
Positive Control: NPY (porcine)
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 5 mM $MgCl_2$ and 0.1% BSA at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the NPY₁ binding site.

Literature Reference:

Martel, J.C., Fournier, S. S., et al. Quantitative Autoradiographic Distribution of [^{125}I]-BH NPY Receptor Binding Sites in Rat Brain. Comparison with [^{125}I]-PYY Receptor Sites. *Neuroscience.* **36 (1):** 255-283 (1990) with modifications.

NEUROPEPTIDE Y (NPY), NON-SELECTIVE
BINDING ASSAY
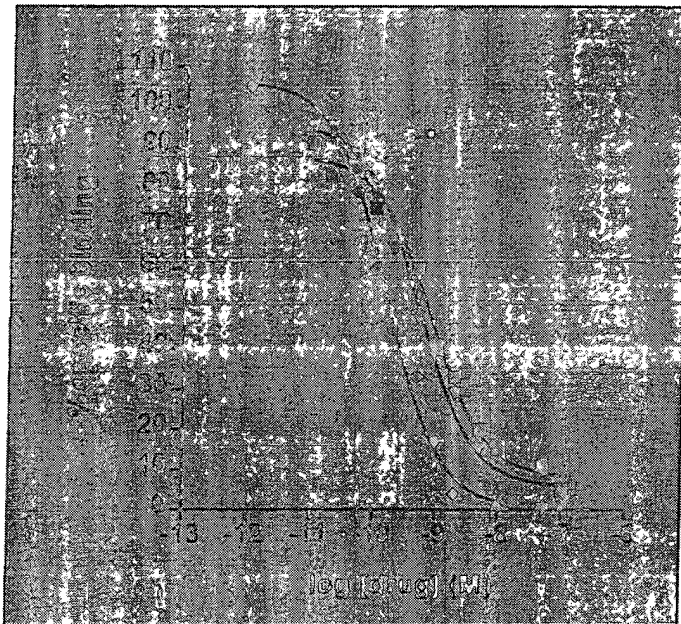
Reference Compounds	Ki (nM)
◆ PYY	0.1
▼ NPY(13-36)	0.5
■ NPY (porcine)	1.3

Assay Characteristics:

K_D (binding affinity):	0.20 nM
B_{max} (receptor number):	89 fmol/mg tissue (wet weight)

Materials and Methods:

|	
Receptor Source:	Bovine hippocampal membranes
Radioligand:	[^{125}I]Polypeptide Y (PYY) (4000 Ci/mmol)
	Final ligand concentration - [0.025 nM]
Non-specific Determinant:	Neuropeptide Y (porcine) - [1.0 μM]
Reference Compound:	Neuropeptide Y (porcine)
Positive Control:	Neuropeptide Y (porcine)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 5 mM $MgCl_2$, 0.1 mg/ml soybean trypsin inhibitor, 0.1% BSA, and 0.25 mg/ml bacitracin at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the neuropeptide Y binding site.

Literature Reference:

Chang, R.S, Lotti, V.J., Chen, T.B. et al. Neuropeptide Y Binding Sites in Rat Brain Labeled with [^{125}I]Bolton-Hunter NPY: Comparative Potencies of Various Polypeptides on Brain NPY Binding and Biological Responses in the Vas Deferens. *Life Science.* **37**: 2111-2122 (1985) with modifications.

Martel, J.C., Fournier, S. S., et al. Quantitative Autoradiographic Distribution of [^{125}I]-BH NPY Receptor Binding Sites in Rat Brain. Comparison with [^{125}I]-PYY Receptor Sites. *Neuroscience.* **36 (1):** 255-283 (1990) with modifications.

NEUROTENSIN
BINDING ASSAY
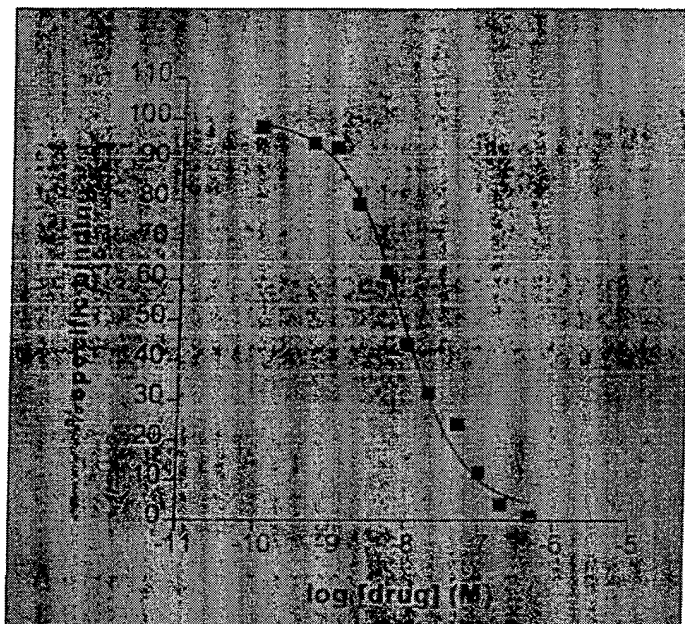
Reference Compounds	Ki (nM)
■ Neurotensin	10.8
Neurotensin (8-13)	7.7
Neurotensin (10-13)	288.0

Assay Characteristics:

K_0 (binding affinity):	9.8 nM
B_{max} (receptor number):	15 fmol/mg tissue (wet weight)

Materials and Methods:

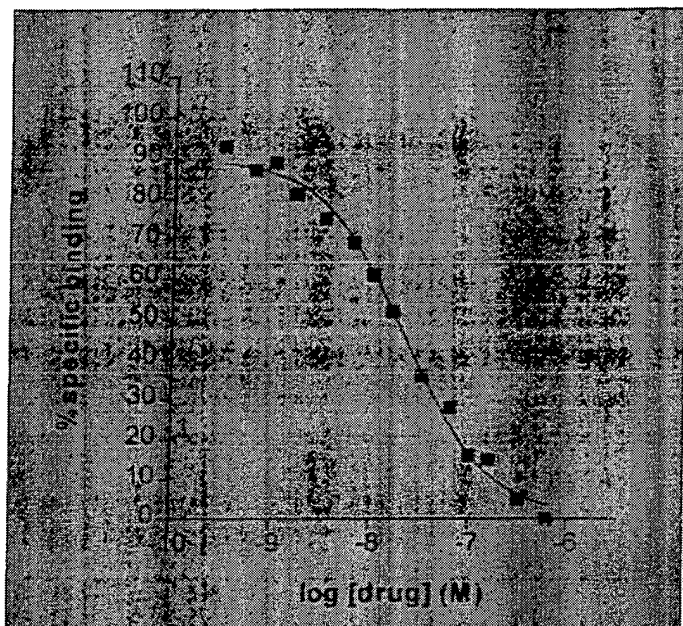|	
Receptor Source;	Rat forebrain membranes
Radioligand:	[^{3}H]Neurotensin (70-120 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	1.0 μM Neurotensin
Reference Compound:	Neurotensin
Positive Control:	Neurotensin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 0.04% bacitracin, 0.1% BSA and 1 mM Na$_2$EDTA for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interaction of test compound with the neurotensin binding site.

Literature Reference:

Goedert, M., Pittaway, K., Williams, B. J. and Emson, P. C. Specific Binding of Tritiated Neurotensin to Rat Brain Membranes: Characterization and Regional Distribution. *Brain Research.* **304:** 71-81 (1984) with modifications.

Gully, D., Canton, M., et al. Biochemical and Pharmacological Profile of a Potent and Selective Nonpeptide Antagonist of the Neurotensin Receptor. *Proc. Nat'l Acad. Sci.* **90:** 65-69 (1993).

NEUROPEPTIDE, NPY$_2$ (HUMAN)
BINDING ASSAY
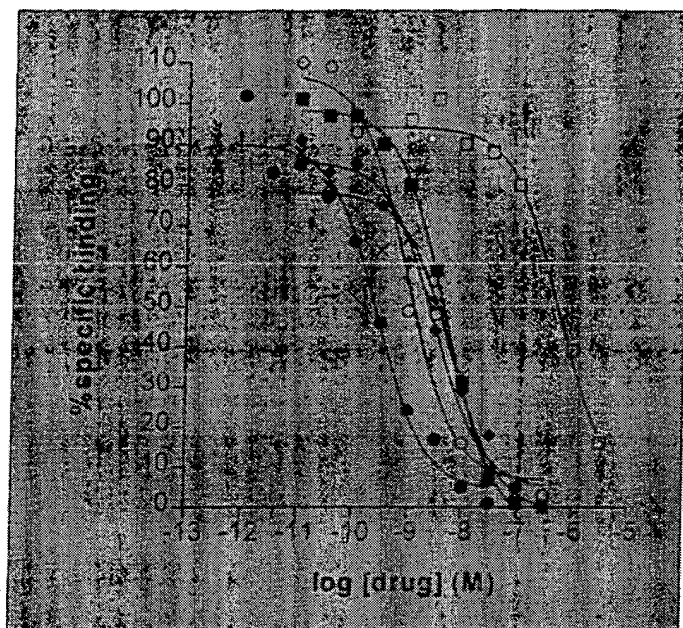
Reference Compounds	Ki (nM)
● PYY	0.1
○ NPY (18-36)	0.4
◆ NPY (3-36)	1.3
■ NPY (rat, human)	1.5
× NPY (13-36)	2.7
□ [Leu^{31}, Pro^{34}]-NPY	244.4

Assay Characteristics:

K$_D$ (binding affinity):	19.6 pM
B$_{max}$ (receptor number):	95 fmol/mg protein

Materials and Methods:

Receptor Source:	KAN-TS Cells
Radioligand:	[^{125}I]Peptide YY (PYY) (4000 Ci/mmol)
	Final ligand concentration - [0.025 nM]
Non-specific Determinant:	NPY (human, rat) - [0.1 μM]
Reference Compound:	NPY (human, rat)
Positive Control:	NPY (human, rat)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 5 mM MgCl$_2$ and 0.1% BSA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the NPY$_2$ binding site.

Literature Reference:

Martel, J.C., Fournier, S. S., et al. Quantitative Autoradiographic Distribution of [^{125}I]-BH NPY Receptor Binding Sites in Rat Brain. Comparison with [^{125}I]-PYY Receptor Sites. *Neuroscience.* **36 (1):** 255-283 (1990) with modifications.

NICOTINIC NEURONAL (α-BUNGAROTOXIN INSENSITIVE SITE) (FORMERLY GANGLIONIC) BINDING ASSAY
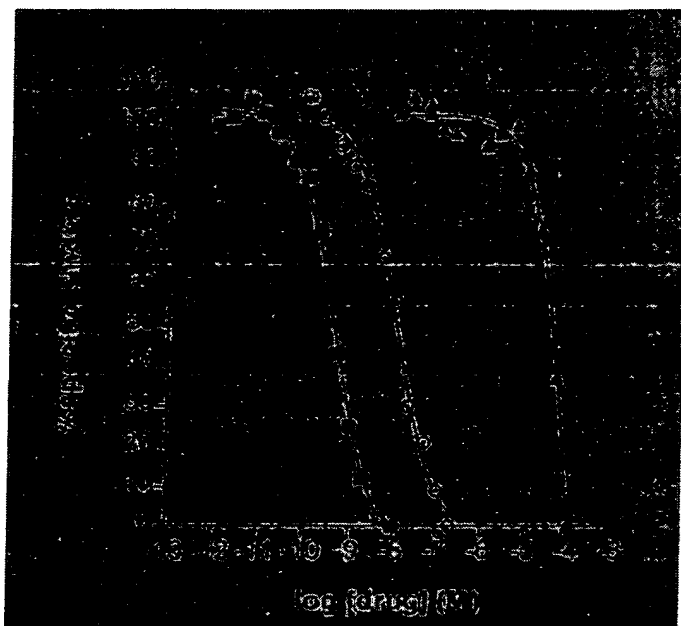
Reference Compounds	Ki (nM)
■ (+/-) Epibatidine	0.06
○ Nicotine Sulfate	4.10
▲ Atropine Sulfate	70,000

Assay Characteristics:

K_D (binding affinity):	63 pM
B_{max} (receptor number):	3.4 fmol/mg tissue

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]Epibatidine (30-60 Ci/mmol)
	Final ligand concentration - [0.1 nM]
Non-specific Determinant:	(+/-) Epibatidine 2HCl- [20 nM]
Reference Compound:	(+/-) Epibatidine 2HCl
Positive Control:	(+/-) Epibatidine 2HCl
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 120 mM NaCl, 5.0 mM KCl, 2.0 mM CaCl$_2$, 1.0 mM MgCl$_2$ and 3.0 μM atropine sulfate at 4°C for 150 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the nicotinic, ganglionic binding site.

Literature Reference:

Luetje, C.W., Patrick, J., and Seguela, P. Nicotine Receptors in the Mammalian Brain. *FASEB Jrnl.* **4:** 2753-2760 (1990) with modificiations.

Perry, D.C. and Kellar, K.J. [^{3}H]Epibatidine Labels Nicotinic Receptors in Rat Brain: An Autoradiographic Study. *Jrnl. Pharmacol. Exp. Ther.* **275:** 1030-1034 (1995) with modifications.

Fisher, M., Huanfu, D. Shen, T.Y. and Guyenet, P.G. Epibatidine, An Alkaloid from the Poison Frog Epidedobates Tricolor, is a Powerful Ganglionic Depolarizing Agent. *Jrnl. Pharmacol. Exp. Ther.* **2:** 702-707 (1994).

NEUROTENSIN (HUMAN RECOMBINANT)
BINDING ASSAY
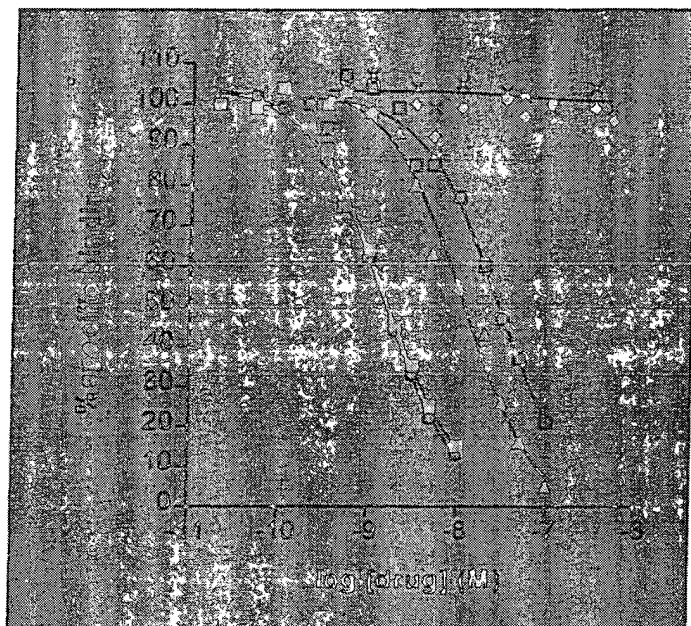
Reference Compounds	Ki (nM)
◨ Acetyl-Neurotensin$_{8-13}$	0.9
○ Neurotensin	0.9
▲ Neuromedin N	6.4
▢ Neurotensin$_{9-13}$	12.4
◆ Neurotensin$_{1-11}$	>1000.0
✕ Neurotensin$_{1-8}$	>1000.0

Assay Characteristics:

K_D (binding affinity):	0.15 nM
B_{max} (receptor number):	1 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{125}I]Neurotensin (2000 Ci/mmol)
	Final ligand concentration - [0.1 nM]
Non-specific Determinant:	Acetyl-Neurotensin$_{8-13}$ - [0.3 uM]
Reference Compound:	Acetyl-Neurotensin$_{8-13}$
Positive Control:	Acetyl-Neurotensin$_{8-13}$
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 0.2% BSA for 60 minutes at 4°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interaction of test compound with the cloned neurotensin binding site.

Literature Reference:

Goedert, M., Pittaway, K., Williams, B. J. and Emson, P. C. Specific Binding of Tritiated Neurotensin to Rat Brain Membranes: Characterization and Regional Distribution. *Brain Research.* **304:** 71-81 (1984) with modifications.

Gully, D., Canton, M., et al. Biochemical and Pharmacological Profile of a Potent and Selective Nonpeptide Antagonist of the Neurotensin Receptor. *Proc. Nat'l Acad. Sci.* **90:** 65-69 (1993).

NOREPINEPHRINE TRANSPORTER
BINDING ASSAY
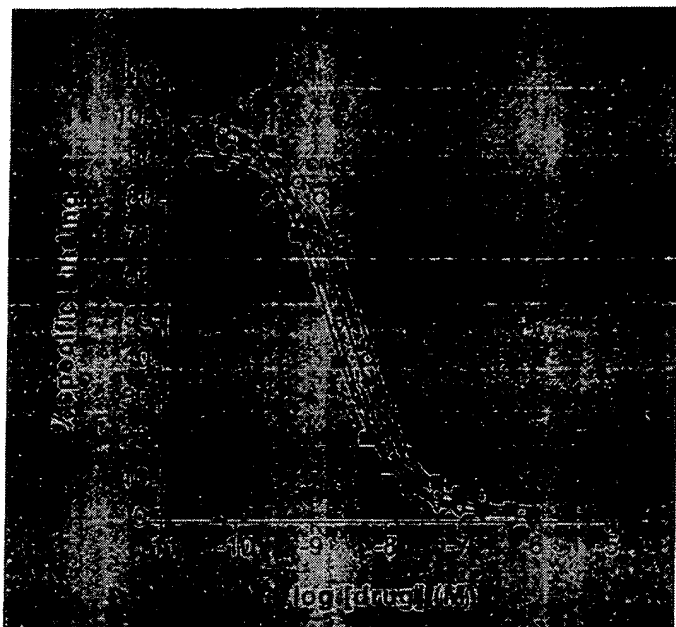
Reference Compounds	Ki (nM)
■ Mazindol	0.8
▽ DMI	0.7
◆ Protriptyline	1.7
○ Nisoxetine	2.4

Assay Characteristics:

K_D (binding affinity):	0.8 nM
B_{max} (receptor number):	10.5 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Nisoxetine (60-85 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Desipramine (DMI) - [1.0 uM]
Reference Compound:	Desipramine (DMI), Imipramine, amitriptyline, or nisoxetine
Positive Control:	Desipramine (DMI), Imipramine, amitriptyline, or nisoxetine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4), containing 300 mM NaCl and 5mM KCl at 0^0 - 4^0C for 4 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interaction of test compound with the norepinephrine uptake site.

Literature Reference:

Raisman, R., Sette, M., Pimoule, C.,et al. High Affinity [^{3}H]Desipramine Binding in the Peripheral and Central Nervous System: A Specific Site Associated with the Neuronal Uptake of Noradrenaline. *Eur. Jrnl. Pharmacol.* **78:** 345-351 (1982) with modifications.

Langer, S. Z., Raisman, R. and Briley, M. [^{3}H]DMI Binding is Associated with Neuronal Noradrenaline Uptake in the Periphery and the Central Nervous System. *Eur. Jrnl. Pharmac.* **72:** 423 (1981).

NITRIC OXIDE SYNTHASE, NOS (CONSTITUTIVE, NEURONAL)
BINDING ASSAY
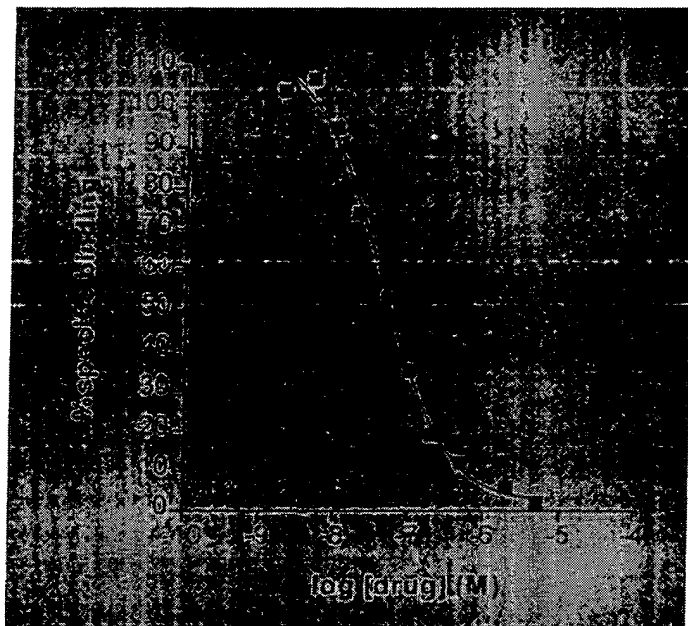
Reference Compounds	IC_{50} (nM)
■ NOARG	55.4

Assay Characteristics:

K_D (binding affinity):   25 nM

B_{max} (receptor number):

Materials and Methods:

Tissue Source: Rat brain membranes

Radioligand: $[^3H]$-L-N^G-Nitro-Arginine [NOARG] (55 Ci/mmol)
Final Concentration - [5.0 nM]

Non-specific Determinant: NOARG - [100 μM]

Reference Compound: NOARG

Positive Control: NOARG

Incubation Conditions: Reactions carried out in 50 mM TRIS-HCl (pH 7.4) for 60 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with NOS enzyme assay.

Literature Reference: Michel, A.D., Phul, R.K., Stewart, T.L., and Humphrey, P.A. Characterization of the Binding of $[^3H]$-L-N^G-Nitro-Arginine in Rat Brain. *Brit. Jrnl. Pharmacol.* **109:** 287-288 (1993) with modifications.

OPIATE, DELTA₁
BINDING ASSAY
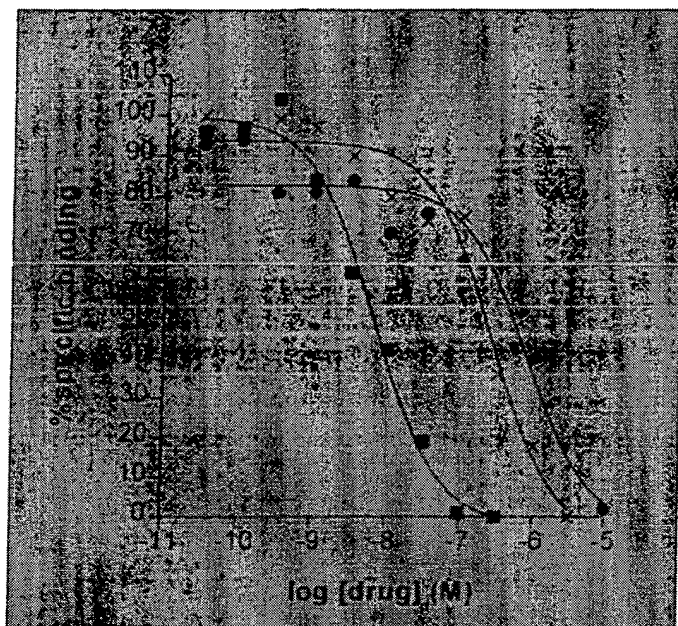
Reference Compounds	Ki (nM)
■ DPDPE	4.5
× Naloxone	158.0
● DAMGO	1022.0

Assay Characteristics:

K_D (binding affinity):	2.1 nM
B_{max} (receptor number):	4.5 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[³H]Deltorphin II (30-60 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	DPDPE - [1.0 µM]
Reference Compound:	DPDPE
Positive Control:	DPDPE
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) for 90 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the delta opiate binding site.
Literature Reference:	Akiyama, K., Gee, K.W., Mosberg, K.W., Yamamura, H.I. Characterization of [³H]DPDPE Binding to Delta Opiate Receptors in the Rat Brain and Neuroblastomaglioma Hybrid Cell Line (NG 108-115). *Proc. Nat'l Acad. Sci.* **82:** 2543 (1985) with modifications.
	Sofuoglu, M., Portoghese, P.S., and Takemori, A.E. δ-Opioid Receptor Binding in Mouse Brain: Evidence for Heterogeneous Binding Sites. *Eur. Jrnl. Pharm.* **216:** 273-277 (1992).

NUCLEAR TESTOSTERONE
BINDING ASSAY
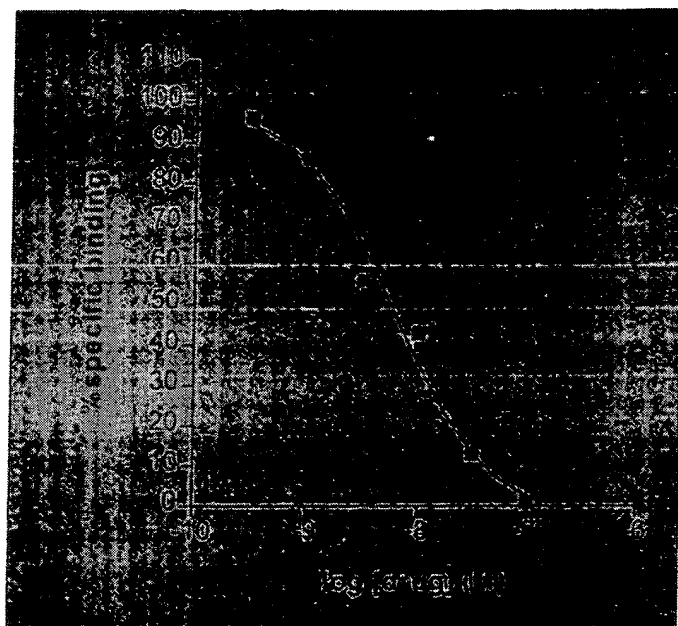
Reference Compounds	Ki (nM)
■ R1881	2.3

Assay Characteristics:

K_D (binding affinity):	1.0 nM
B_{max} (receptor number):	2.2 fmol/mg tissue (initial wet weight)

Materials and Methods:

Receptor Source:	Testosterone pre-treated Rat prostate
Radioligand:	[^{3}H]Methyltrienolone (R1881) (70-87 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Methyltrienolone (R1881) - [1.0 μM]
Reference Compound:	Methyltrienolone (R1881)
Positive Control:	Methyltrienolone (R1881)
Incubation Conditions:	Reactions are carried out in 25 mM HEPES buffer (pH 7.4) containing, 10 mM sodium molybdate, 0.5 mM DTT, 250 mM sucrose, 2.5 mM MgCl$_2$, and 1 mM PMSF at 0-4°C for 18 hours. The reaction is terminated by rapid vacuum filtration onto GF/C filters and the radioactivity bound to the filter is compared to control values in order to ascertain any interactions of test compound with the testosterone binding site.

Literature Reference:

OPIATE, KAPPA₁ BINDING ASSAY
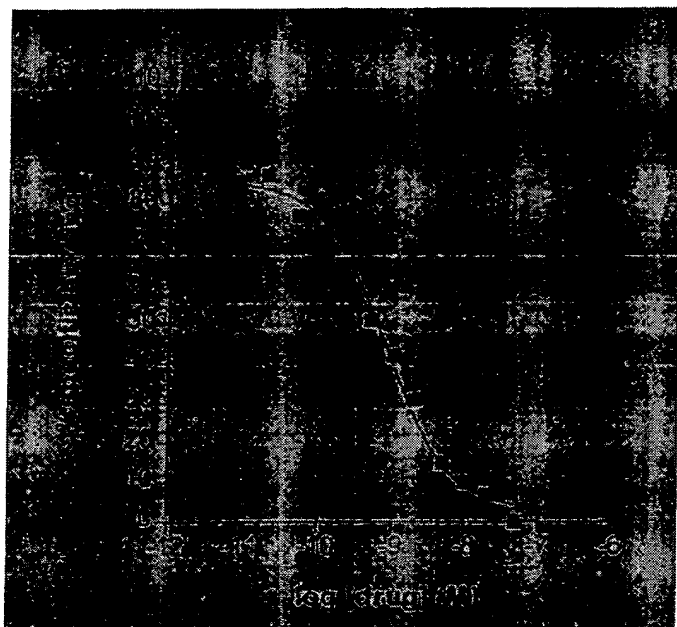
Reference Compounds	Ki (nM)
■ U-69593	0.2

Assay Characteristics:

K_D (binding affinity):   0.75 nM
B_{max} (receptor number):   3.0 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Guinea pig cerebellar membranes
Radioligand: [³H]U-69593 (40-60 Ci/mmol)
    Final ligand concentration - [0.75 nM]
Non-specific Determinant: U-69593 - [1.0 μM]
Reference Compound: U-69593
Positive Control: U-69593
Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.4) at 30°C for 120 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the kappa opiate binding site.

Literature Reference:

Lahti, et al. [³H]U-69593: A Highly Selective Ligand for the Opioid Kappa Receptor. *Eur. Jrnl. Pharmac.* **109**: 281-284 (1985) with modifications.

Rothman, R.B., et al. Interaction of Opioid Peptides and Other Drugs with Multiple Kappa Receptors in Rat and Human Brain. Evidence for Species Differences. *Peptides.* **13**: 977-987 (1992).

Kinouchi, K. and Pasternak, G.W. Evidence for κ Opioid Receptor Multiplicity in the Guinea Pig Cerebellum. *Eur. Jrnl. Pharmac.* **207**: 135-141 (1991).

OPIATE, DELTA$_2$ (HUMAN RECOMBINANT)
BINDING ASSAY
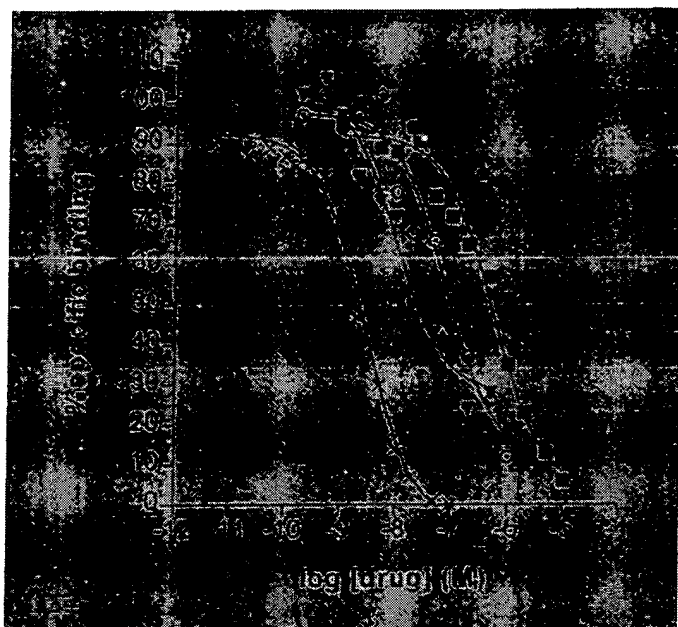
Reference Compounds		Ki (nM)
✕ Naltriben		1.1
▼ DPDPE		3.8
○ Naltrexone		29.3
■ Naloxone		200.4

Assay Characteristics:

K_D (binding affinity):	0.3 nM
B_{max} (receptor number):	6 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{3}H]-Naltrindole (30-50 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specific Determinant:	Naltriben - [3.0 μM]
Reference Compound:	Naltriben
Positive Control:	Naltriben
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM MgCl$_2$, at 25^{0}C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned delta$_2$ binding site.

Literature Reference:	Malatynska, et. al., *NeuroReport*, **6**: 613-616 (1995), with modifications.

Accession Number:	Genbank U10504.

OPIATE, MU
BINDING ASSAY
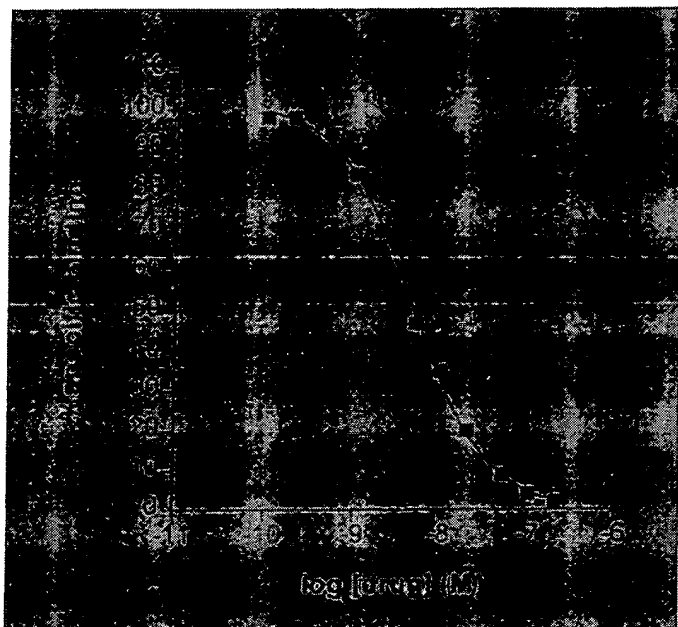
Reference Compounds	Ki (nM)
DAMGO	0.8
Cyclazocine	1.6
▬ Naloxone	3.7
DADLE	22.2
DSLET	87.0
U-50488	450.0
DPDPE	1,130.0

Assay Characteristics:

K_D (binding affinity):	3.7 nM
B_{max} (receptor number):	7.3 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO) (30-60 Ci/mmol) Final ligand concentration - [1.0 nM]
Non-specifc Determinant:	Naloxone - [1.0 µM]
Reference Compound:	Naloxone
Positive Control:	Naloxone
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the opiate mu binding site.

Literature Reference:

Gillan, M.G.C., and Kosterlitz, H.W. Spectrum of the Mu, Delta, and Kappa Binding Sites in Homogenates of Rat Brain. *Brit. Jrnl. Pharmacol.* **77**: 461 (1982) with modifications.

Goldstein, A. and Naidu, A. Multiple Opioid Receptors: Ligand Selectivity Profiles and Binding Site Signatures. *Mol. Pharmacol.* **36**: 265-272 (1989).

OPIATE, KAPPA (HUMAN RECOMBINANT)
BINDING ASSAY
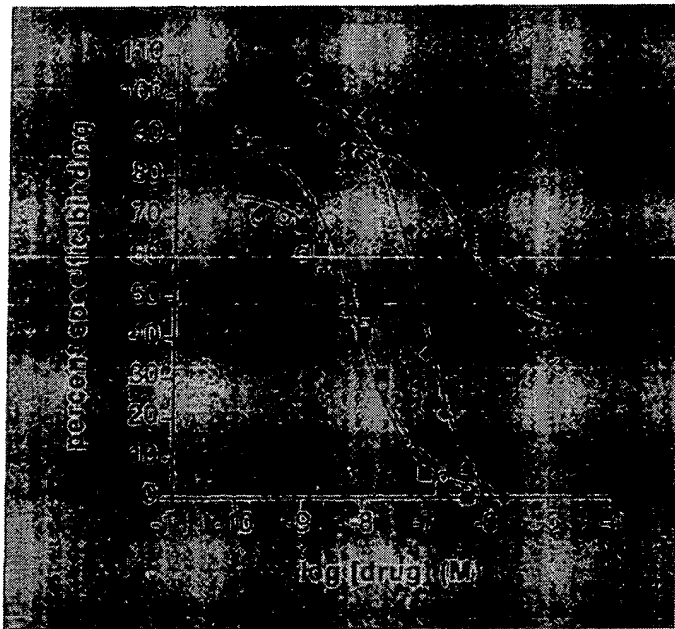
Reference Compound	Ki (nM)
■ Naloxone	2.4
○ U69593	2.8
◆ Naltriben	25.3
× DAMGO	111.0

Assay Characteristics:

K_D (binding affinity):	0.26 nM
B_{max} (receptor number):	1.5 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in CHO cells
Radioligand:	[^{3}H]-diprenorphine (30-50 Ci/mmol)
	Final ligand concentration - [0.6 nM]
Non-specific Determinant:	Naloxone - [10 μM]
Reference Compound:	Naloxone
Positive Control:	Naloxone
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl$_2$, 1 mM EDTA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned kappa binding site.

Literature Reference:	Simonin, F., et. al., Kappa-Opioid Receptor in Humans: cDNA and Genomic Cloning, Chromosomal Assignment, Functional Expression, Pharmacology and Expression Pattern in the Central Nervous System. *Proc. Natl. Acad. Sci. U.S.A.* **92(15)**: 1431-1437, 1995, with modifications.

Accession number:	GenBank U17298.

OPIATE, NON-SELECTIVE
BINDING ASSAY
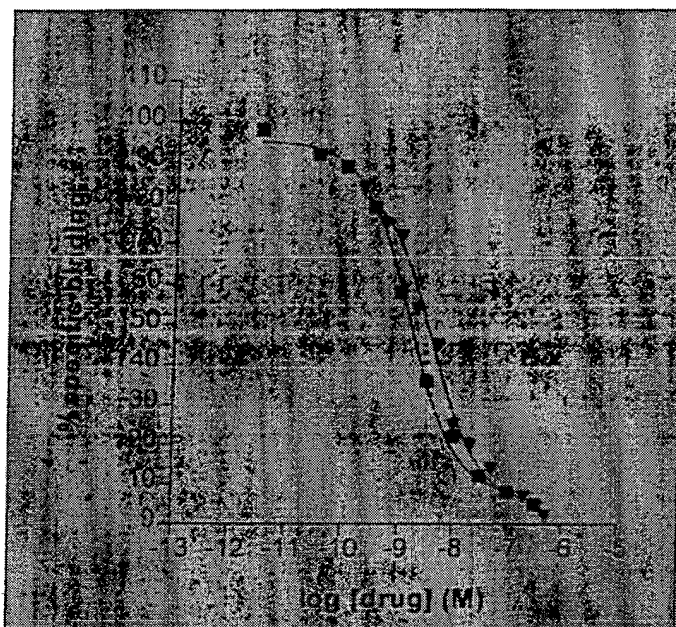
Reference Compounds	Ki (nM)
■ Naloxone	1.5
▼ Morphine	3.3

Assay Characteristics:

K_0 (binding affinity):	2.0 nM
B_{max} (receptor number):	10 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Naloxone (40-60 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Naloxone - [1.0 μM]
Reference Compound:	Naloxone
Positive Control:	Naloxone
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the opiate non-selective binding site.

Literature Reference:

Pert, C. and Snyder, S.H. Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium. *Mol. Pharmacol.* **19:** 868-879 (1974) with modifications.

Pert, C. and Snyder, S. H. Regional Distribution of Opiate Receptor Binding in Monkey and Human Brain. *Nature.* **245:** 447-450 (1973).

OPIATE, MU (HUMAN RECOMBINANT)
BINDING ASSAY
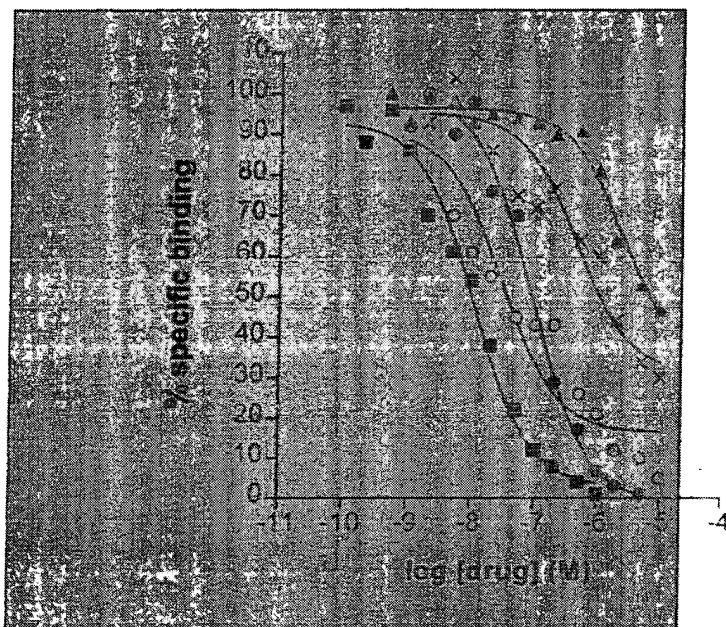
Reference Compounds		Ki (nM)
▣	Naloxone	3.1
○	DAMGO	8.3
●	Naltriben	24.9
×	DPDPE	121.7
▲	U69593	698.5

Assay Characteristics:

K_D (binding affinity):   0.23 nM

B_{max} (receptor number):   3 pmol/mg protein

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO cells

Radioligand: [^{3}H]-Diprenorphine (30-50 Ci/mmol)
Final ligand concentration - [0.6 nM]

Non-specific Determinant: Naloxone - [10 μM]

Reference Compound: Naloxone

Positive Control: Naloxone

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM $MgCl_2$, at 25°C for 150 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned mu binding site.

Literature Reference: Malatynska, et. al., *NeuroReport*, **6**: 613-616 (1995), with modifications.

Accession Number: Genbank L25119.

PLATELET ACTIVATING FACTOR (PAF)
BINDING ASSAY
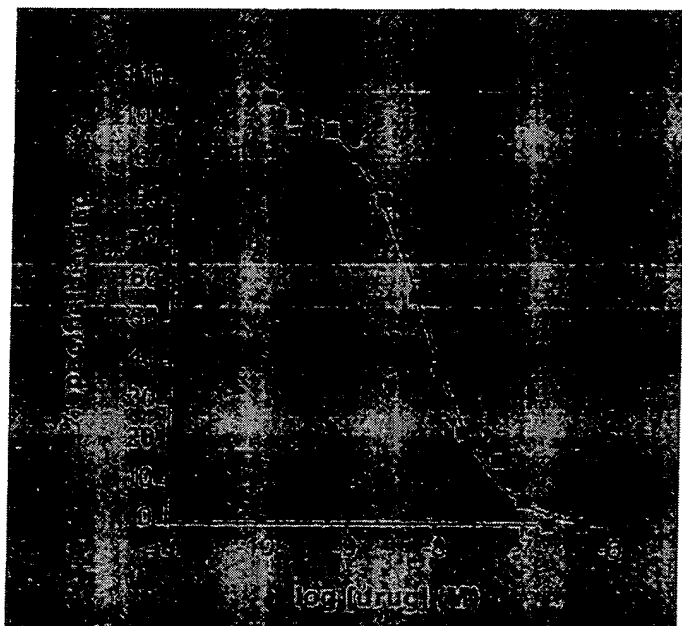
Reference Compounds ....Ki (nM)
■ C$_{16}$-PAF   4.8

Assay Characteristics:

K$_D$ (binding affinity):   1.7 nM
B$_{max}$ (receptor number):   11.5 pmol/mg protein

Materials and Methods:

Receptor Resource: Rabbit platelets
Radioligand: [^{3}H]Hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, (PAF)
(60-180 Ci/mmol)
Final ligand concentration - [1.0 nM]
Non-specific Determinant: C$_{16}$-PAF - [1.0 uM]
Reference Compound: C$_{16}$-PAF
Positive Control: C$_{16}$-PAF
Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.0) containing 0.25% BSA at 0°C for 2 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the platelet activating factor binding site.

Literature Reference:

Hwang, H., Lee, C., Cheah, M. and Shen, T. Y. Trans-2,5- Bis-(3,4,5-trimethoxyphenyl) Tetrahydrofuran. An Orally Active Specific and Competitive Receptor Antagonist of Platelet Activating Factor. *Jrnl. Biol. Chem.* **260(29):** 15639-45 (1985) with modifications.

Hwang, S-B., et al. Specific Receptor Sites for 1-*O*-Alkyl-2-*O*-aceyl-*sn*-glycero-3-phosphocholine (PAF) on Rabbit Platelet and Guinea Pig Smooth Muscle Membranes. *Biochemistry.* **22:** 4756-4763 (1983).

OXYTOCIN
BINDING ASSAY
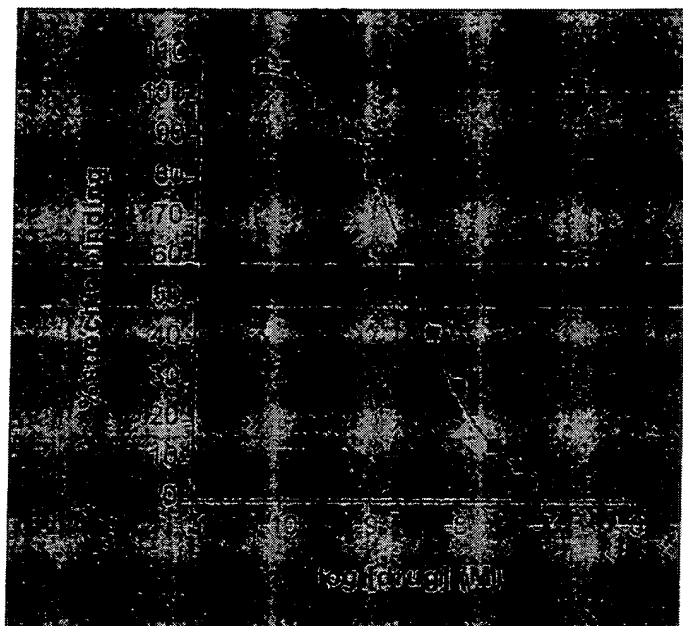
Reference Compounds	Ki (nM)
[Arg⁸]-Vasopressin (AVP)	1.0
[Thr⁴, Gly⁷]-Oxytocin	1.2
■ Oxytocin	1.5
dDAVP	19.2

Assay Characteristics:

K_D (binding affinity):	1.2 nM
B_{max} (receptor number):	10.8 fmol/mg protein

Materials and Methods:

|	
Receptor Source:	Rat uterus membranes
Radioligand:	[³H]-Oxytocin (30-60 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Oxytocin - [1.0 µM]
Reference Compound:	Oxytocin
Positive Control:	Oxytocin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 5 mM MgCl2 and 0.1% BSA at 22°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the oxytocin binding site.
Literature Reference:	Pettibone, D.J., Woyden, C.J., and Totaro, J.A. Identification of Functional Oxytocin Receptors in Lactating Rat Mammary Gland *in vitro*. *Eur. Jrnl. Pharmacol.* **188**:235-242 (1990) with modifications.
	Fuchs, A.R., Behrens, O., et al. Oxytocin and Vasopressin Receptors in Bovine Endometrium and Myometrium During the Estrous Cycle and Early Pregnancy. *Endocrinology.* **127(2)**: 629-636 (1990).

POTASSIUM CHANNEL, Ca²⁺ ACTIVATED, VOLTAGE-INSENSITIVE BINDING ASSAY
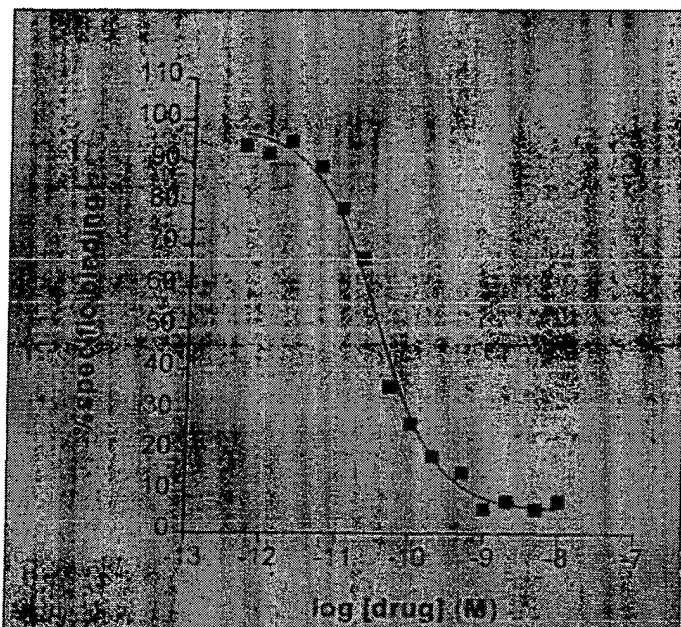
Reference Compounds	Ki (nM)
■ Apamin	0.03
TBPS	>10,000
Picrotoxin	>10,000
Glibenclamide	>10,000
Charybdotoxin	>10,000

Assay Characteristics:

K_D (binding affinity):	0.07 nM
B_{max} (receptor number):	0.8 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{125}I]Apamin (2200 Ci/mmol)
	Final ligand concentration - [0.05 nM]
Non-specific Determinant:	Apamin - [100 nM]
Reference Compound:	Apamin
Positive Control:	Apamin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 0.1% BSA, 5 mM KCl at 0-4°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the apamin binding site.

Literature Reference:

Seager, M., Marqueze, B. and Couraud, F. Solubilization of the Apamin Receptor Associated with a Calcium-Activated Potassium Channel From Rat Brain. *Jrnl. Neuroscience.* **7(2):** 565-570 (1987) with modifications.

Habermann, E., and Fisher, K. Bee Venom (Apamin): Iodine Labeling and Characterization of Binding Sites. *Eur. Jrnl. Pharmac.* **94:** 355-364 (1979).

POTASSIUM CHANNEL, ATP-SENSITIVE
BINDING ASSAY
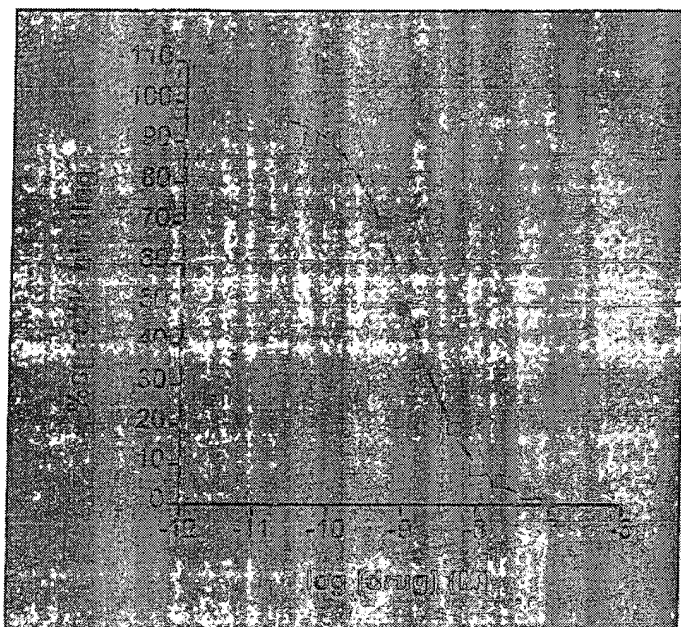
Reference Compounds	Ki (nM)
□ Glibenclamide	0.6
Apamin	>10,000
Nitrendipine	>10,000
TBOB	>10,000
Tolbutamide	>10,000
Chlorpropamide	>10,000

Assay Characteristics:

K_D (binding affinity):   0.25 nM

B_{max} (receptor number):   6.1 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [^{3}H]Glibenclamide (40-70 Ci/mmol)
Final ligand concentration - [0.2 nM]

Non-specific Determinant: Glibenclamide - [0.1 μM]

Reference Compound: Glibenclamide

Positive Control: Glibenclamide

Incubation Conditions: Reactions are carried out in 50 mM phosphate buffer (pH 7.7) at 25°C for 120 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the glibenclamide binding site.

Literature Reference: Geisen, K., Hitzel, V., Okomonopoulos, R., Punter, J., Weyer, R. and Summ, H. Inhibition of ^{3}H-Glibenclamide Binding to Sulfonylurea Receptors by Oral Antibiotics. *Arzneim.-Forsch./Drug Res.* **35(1):** 707-712 (1985) with modifications.

Bernardi, H., Fosset, M. and Lazdunski, M. Characterization, Purification, and Affinity Labeling of the Brain [^{3}H]-Glibenclamide Binding Protein, a Putative Neuronal ATP-Regulated K^+ Channel. *Proc. Nat'l Acad. of Sci.* **85:** 9816-9820 (1988).

PROGESTERONE
BINDING ASSAY
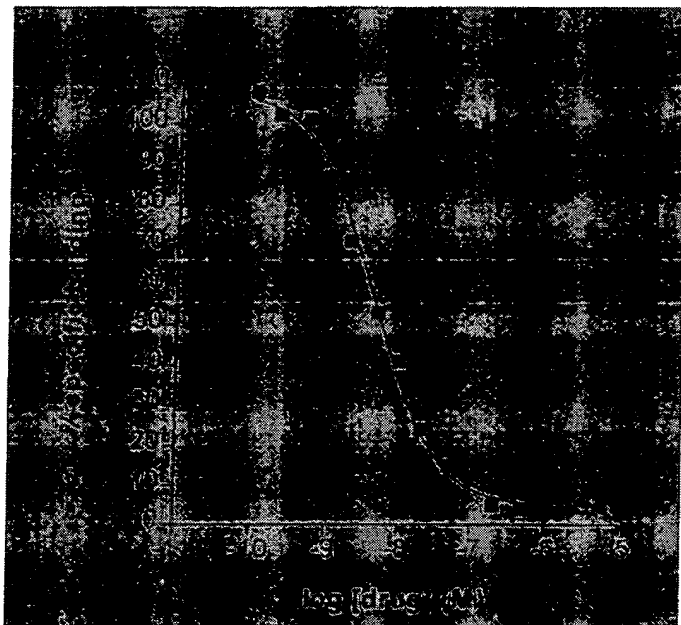
Reference Compounds	Ki (nM)
■ Promegestone	4.3
Corticosterone	320.0
Estradiol	325.0
Testosterone	440.0
Estriol	5,800.0

Assay Characteristics:

K_D (binding affinity):   6.0 nM

B_{max} (receptor number):   152 fmol/mg protein

Materials and Methods:

|	
Receptor Source:	Bovine uterus membranes
Radioligand:	[^{3}H]Promegestone (70-87 Ci/mmol)
	Final ligand concentration - [0.7 nM]
Non-specific Determinant:	Promegestone - [1.0 µM]
Reference Compound:	Promegestone
Positive Control:	Promegestone
Incubation Conditions:	Reactions are carried out in 10 mM TRIS-HCl (pH 7.4) containing 1.5 mM EDTA, 1.0 mM DTT, and 25.0 mM sodium molybdate at 0-4°C for 18 hours. The reaction is terminated by the addition of dextran coated charcoal and incubated for 20 minutes at 0-4°C. The reaction mixture is then centrifuged and the radioactivity bound in the supernatant is assessed and compared to control values in order to ascertain any interactions of test compounds with the progesterone binding site.

Literature Reference:

Haji, et al. Age-Related Changes in the Concentrations of Cytosol Receptors for Sex Steroids in the Hypothalamus and Pituitary Gland of the Rat. *Brain Research.* **204**: 373-386 (1980) with modifications.

Traish, A.M., Muller, R.E., et al. Binding of 7α,17α-Dimethyl-19 Nortestosterone (Mibolerone) to Androgen and Progesterone Receptors in Human and Animal Tissues. *Endocrinology.* **118(4):** 1327-1333 (1986).

POTASSIUM CHANNEL, Ca^{2+} ACTIVATED, VOLTAGE-SENSITIVE
BINDING ASSAY
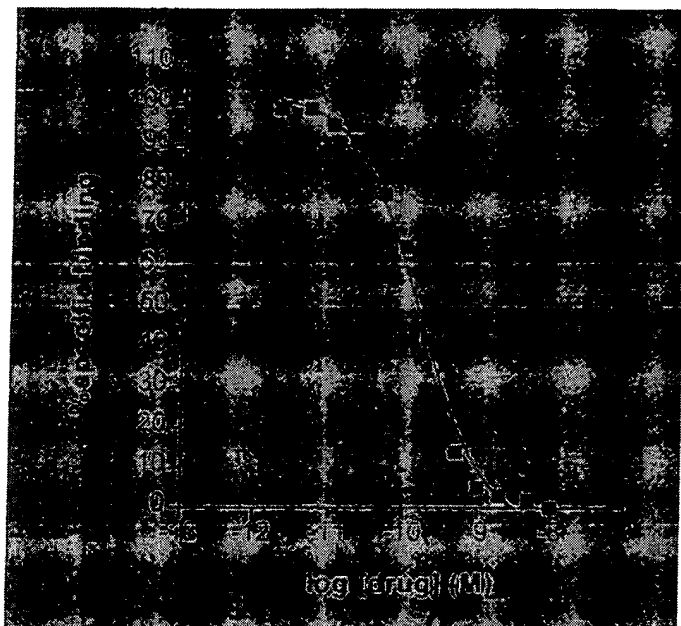
Reference Compounds	Ki (nM)
▣ Charybdotoxin	0.26
Glibenclamide	>1,000
Apamin	>1,000
Nifedipine	>10,000
TBPS	>10,000

Assay Characteristics:

K_D (binding affinity):	0.1 nM
B_{max} (receptor number):	350 fmol/mg protein

Materials and Methods:

Receptor Source:	Rat brain membranes
Radioligand:	[^{125}I]Charybdotoxin (2200 Ci/mmol)
	Final ligand concentration - [0.05 nM]
Non-specific Determinant:	Charybdotoxin - [10 nM]
Reference Compound:	Charybdotoxin
Positive Control:	Charybdotoxin
Incubation Conditions:	Reactions are carried out in 20 mM TRIS-HCl (pH 7.4) containing 100 mM NaCl, and 0.1% BSA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the charybdotoxin binding site.

Literature Reference:

Vazquez, J., et al. Characterization of High Affinity Binding Sites for Charybdotoxin Synaptic Plasma Membranes from Rat Brain. *Jrnl. Biochemistry.* **265**: 15564-15571 (1990) with modifications.

Vazquez, J., et al. Characterization of High Affinity Binding Sites for Charybdotoxin Sarcolemmal Membranes from Bovine Aortic Smooth Muscle. *Jrnl. Biol. Chem.* **264(35)**: 20902-20909.

SEROTONIN, 5HT₁
BINDING ASSAY
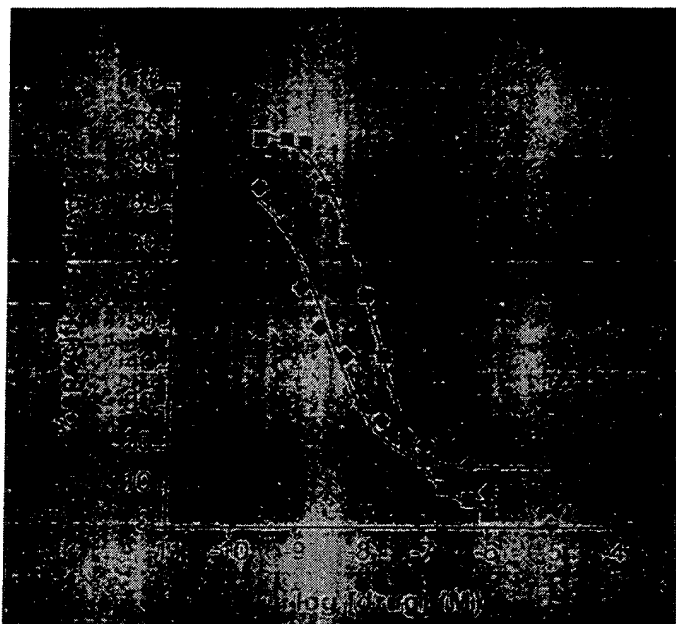
Reference Compounds	Ki (nM)
◆ 5-Carboxytryptamine (5-CT)	2.2
■ 5-Hydroxytryptamine (5-HT)	11.2
5-Methoxytryptamine	45.8
Methysergide	790.0
CGS-12066B	1,055.0

Assay Characteristics:

K_D (binding affinity):	2.8 nM
B_{max} (receptor number):	9.2 fmol/mg protein

Materials and Methods:

Receptor Source: Rat cortical membranes

Radioligand: [³H]Hydroxytryptamine binoxalate (15-30 Ci/mmol)
Final ligand concentration - [3.0 nM]

Non-specific Determinant: Serotonin - [100 μM]

Reference Compound: Serotonin

Positive Control: Serotonin

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT₁ binding site.

Literature Reference: Peroutka, S.J., Snyder, S.H. Multiple Serotonin Receptors: Differential Binding of [³H]-5-HT, [³H]-LSD and [³H]-Spiroperidol. *Mol. Pharmacol.* **16**: 687-699 (1979) with modifications.

Peroutka S.J. and Snyder, S.H. Two Distinct Serotonin Receptors: Regional Variations in Receptor Binding in Mammalian Brain. *Brain Research.* **208**: 339-347 (1981).

Martin, G. R. and Humphrey, P. P. A. Classification Review for 5-HT: Current Perspectives on Classification and Nomenclature. *Neuropharmacol.* **3(3/4)**: 261-273 (1994).

PROTEIN KINASE C, PDBu
BINDING ASSAY
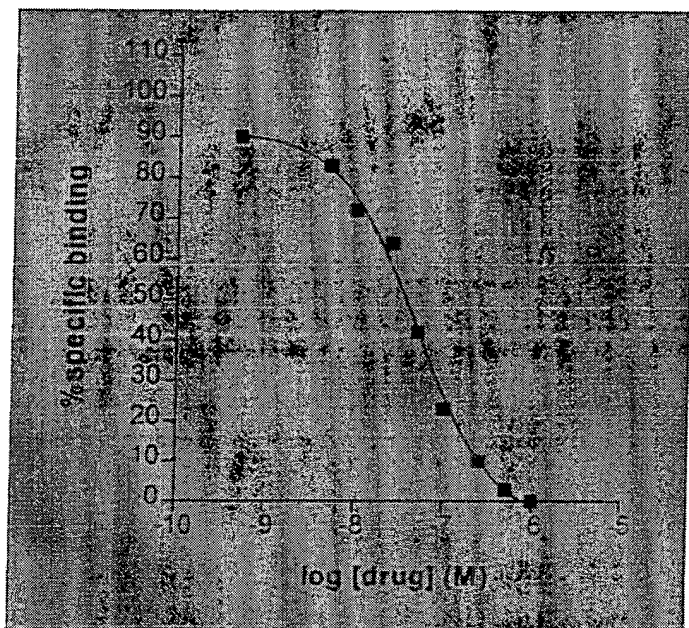
Reference Compounds	Ki (nM)
■ Phorbol, 12, 13 dibutyrate	26.0
Phorbol, 12, 13 diacetate	42.0

Assay Characteristics:

K_D (binding affinity):	13.6 nM
B_{max} (receptor number):	28.4 pmol/mg protein

Materials and Methods:

Receptor Source:	Mouse brain membranes
Radioligand:	[^{3}H]Phorbol ester dibutyrate (PDBu) (10-20 Ci/mmol)
	Final ligand concentration - [4.0 nM]
Non-specific Determinant:	PDBu - [1.0 μM]
Reference Compound:	PDBu
Positive Control:	PDBu
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 1.0% BSA and 0.5 mM $CaCl_2$ at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the phorbol ester binding site.

Literature Reference:

Dunphy, W. G., Delclos, K. B., and Blumberg, P. M. Characterization of Specific Binding of [^{3}H]Phorbol 12, 13-dibutyrate and [^{3}H]Phorbol 12-myristate 13-acetate to Mouse. *Cancer Research.* **40**: 3635-3641 (1980) with modifications.

Delclos, K. B., et al. Specific Binding of Phorbol Ester Tumor Promoters to Mouse Skin. *Cell.* **19**: 1025-1032 (1980).

Driedger, P. E., et al. Specific Binding of Phorbol Ester Promoters. *Proc. Nat'l. Acad. Sci.* **77**: 567-571 (1980).

SEROTONIN, 5HT$_{1A}$ (HUMAN RECOMBINANT)
BINDING ASSAY

Reference Compounds	Ki (nM)
■ 8-OH-DPAT	1.5
□ Serotonin	4.0
× Metergoline	5.3

Assay Characteristics:

K$_D$ (binding affinity):	1.8 nM
B$_{max}$ (receptor number):	370 fmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in HeLa cells
Radioligand:	[^{3}H]-8-OH-DPAT (100 Ci/mmol)
	Final ligand concentration - [0.25 nM]
Non-specific Determinant:	8-OH-DPAT - [1.0 μM]
Reference Compound:	8-OH-DPAT
Positive Control:	8-OH-DPAT
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl, 0.5 mM EDTA, and 0.1% Ascorbic acid at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned 5HT$_{1A}$ binding site.

Literature Reference:

Hoyer, D., Engel, G., et al. Molecular Pharmacology of 5HT$_1$ and 5-HT$_2$ Recognition Sites in Rat and Pig Brain Membranes: Radioligand Binding Studies with [^{3}H]-5HT, [^{3}H]-8-OH-DPAT, [^{125}I]-Iodocyanopindolol, [^{3}H]-Mesulergine and [^{3}H]-Ketanserin. *Eur. Jrnl. Pharmacol.* **118**: 13-23 (1985) with modifications.

Schoeffter, P. and Hoyer, D. How Selective is GR 43175? Interactions with Functional 5-HT$_{1A}$, 5HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ Receptors. *Naunyn-Schmiedeberg's Arch. Pharmac.* **340**: 135-138 (1989) with modifications.

SEROTONIN, 5HT$_{1A}$
BINDING ASSAY
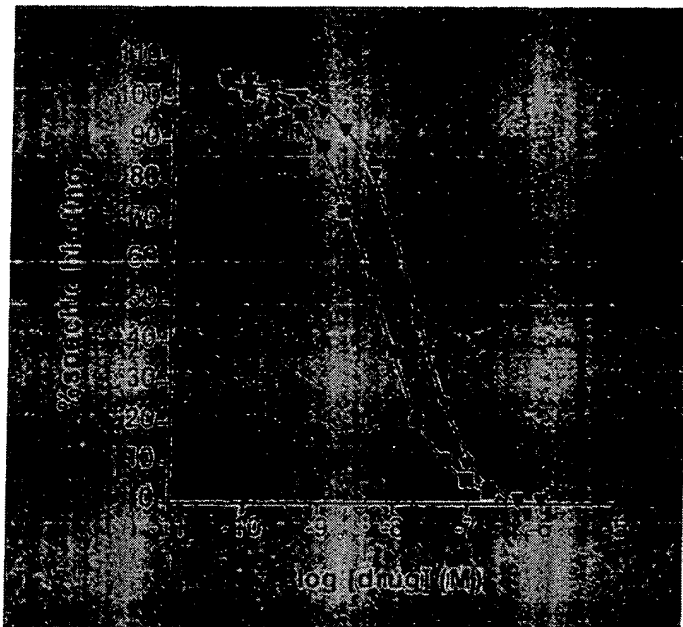
Reference Compounds	Ki (nM)
■ 8-OH-DPAT	2.9
RU 24969	10.0
▼ Serotonin	12.4
Ketanserin	>10,000

Assay Characteristics:

K_D (binding affinity):	2.0 nM
B_{max} (receptor number):	1.626 pmol/mg protein

Materials and Methods:

|	
Receptor Source:	Bovine hippocampal membranes
Radioligand:	[^{3}H]-8-OH-DPAT (100 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Serotonin - [10 μM]
Reference Compound:	8-OH-DPAT
Positive Control:	Serotonin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT$_{1A}$ binding site.

Literature Reference:

Hoyer, D., Engel, G., et al. Molecular Pharmacology of 5HT$_1$ and 5-HT$_2$ Recognition Sites in Rat and Pig Brain Membranes: Radioligand Binding Studies with [^{3}H]-5HT, [^{3}H]-8-OH-DPAT, [^{125}I]-Iodocyanopindolol, [^{3}H]-Mesulergine and [^{3}H]-Ketanserin. *Eur. Jrnl. Pharmacol.* **118**: 13-23 (1985) with modifications.

Schoeffter, P. and Hoyer, D. How Selective is GR 43175? Interactions with Functional 5-HT$_{1A}$, 5HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ Receptors. *Naunyn-Schmiedeberg's Arch. Pharmac.* **340**: 135-138 (1989) with modifications.

SEROTONIN, 5HT$_{1D}$
BINDING ASSAY
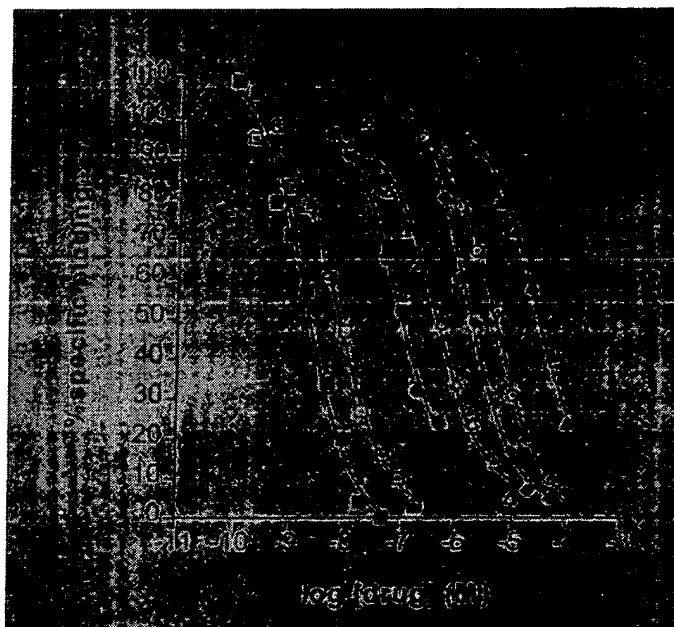
Reference compounds		Ki (nM)
■	5-CT	1.1
□	Serotonin	4.5
●	Methysergide	42.6
	Tryptamine	58.8
	Sumatriptan	60.5
△	Risperidone	233.1
	Methiothepin	295.0
◆	8-OH-DPAT	822.0
	Sertindole	1,013.0
○	Quipazine	2,600.0
▲	Mesulgerine	13,030.0

Assay Characteristics:

K$_D$ (binding affinity):   1.0 nM
B$_{max}$ (receptor number):   60 fmol/mg tissue

Materials and Methods:

Receptor Source: Bovine striatal membranes
Radioligand: [^{3}H] 5-Carboxamidotryptamine (20-70 Ci/mmol)
                       Final ligand concentration - [0.75 nM]
Non-specific Determinant: 5-Carboxamidotryptamine (5-CT) - [1.0 µM]
Reference Compound: 5-Carboxamidotryptamine (5-CT)
Positive Control: 5-Carboxamidotryptamine (5-CT)
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCI (pH 7.7) containing 4 mM CaCl$_2$, 100 nM 8-OH-DPAT, 100 nM Mesulergine, 10 µM pargyline and 0.1% ascorbic acid at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT$_{1D}$ binding site.

Literature Reference:

Waeber C., Schoeffter, Palacios, J.M. and Hoyer, D. Molecular Pharmacology of the 5-HT$_{1D}$ Recognition Sites: Radioligand Binding Studies in Human, Pig, and Calf Brain Membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **337**: 595-601 (1988) with modifications.

SEROTONIN, 5HT$_{1B}$
BINDING ASSAY
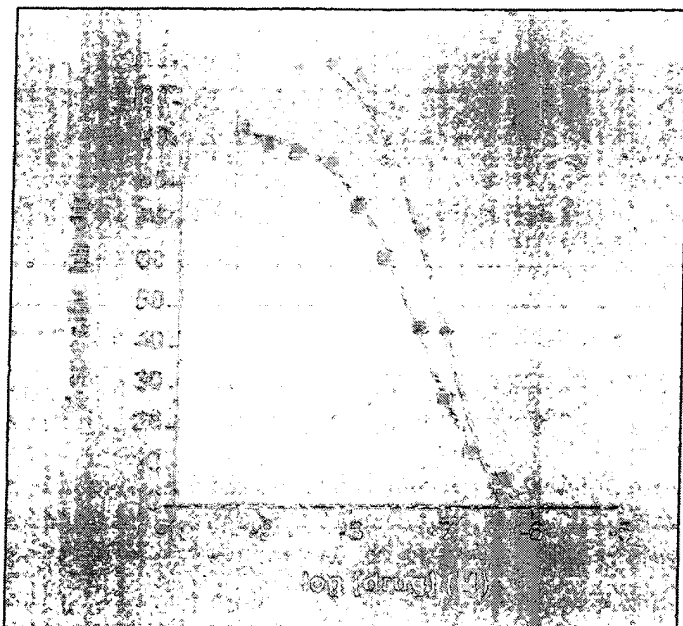
Reference Compounds	Ki (nM)
RU 24989	2.0
▢ Serotonin	13.8
◇ CGS-12066B	42.2
TFMPP	45.0
Chlorophenylpiperazine	51.0
Quipazine	280.0
Phenylpiperazine	415.0

Assay Characteristics:

K$_D$ (binding affinity):	0.12 nM
B$_{max}$ (receptor number):	6.9 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat striatal membranes
Radioligand: [^{125}I]Iodocyanopindolol (2200 Ci/mmol)
Final ligand concentration - [0.15 nM]
Non-specific Determinant: Serotonin - [10 μM]
Reference Compound: Serotonin
Positive Control: Serotonin
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 60 μM (-) isoproterenol at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT$_{1B}$ binding site.

Literature Reference: Hoyer, D., Engel, G., et al. Molecular Pharmacology of 5HT$_1$ and 5-HT$_2$ Recognition Sites in Rat and Pig Brain Membranes: Radioligand Binding Studies with [^{3}H]-5HT, [^{3}H]-8-OH-DPAT, [^{125}I]-Iodocyanopindolol, [^{3}H]-Mesulergine and [^{3}H]-Ketanserin. *Eur. Jrnl. Pharmacol.* **118**: 13-23 (1985) with modifications.

Schoeffter, P. and Hoyer, D. How Selective is GR 43175? Interactions with Functional 5-HT$_{1A}$, 5HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ Receptors. *Naunyn-Schmiedeberg's Arch. Pharmac.* **340**: 135-138 (1989) with modifications.

SEROTONIN, $5HT_{2A}$ ($5HT_2$)
BINDING ASSAY
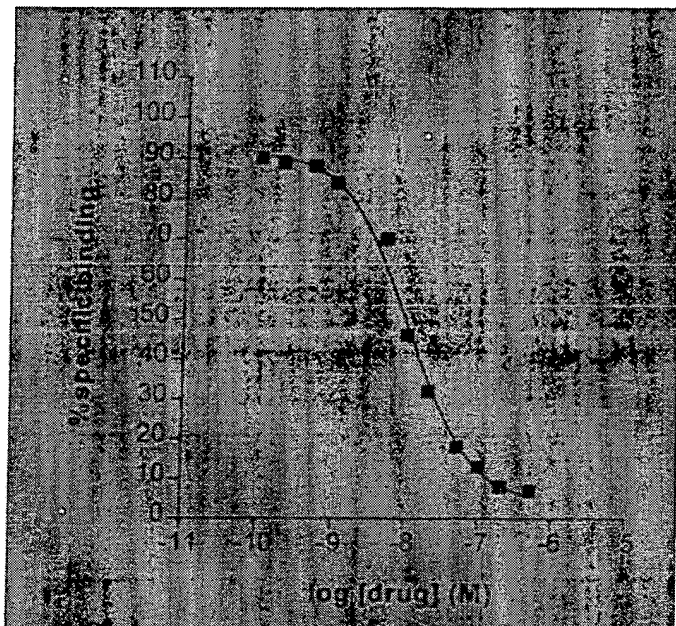
Reference Compounds	Ki (nM)
Ketanserin	0.4
■ Methysergide	1.6
D-LSD	2.1
Serotonin	531.0

Assay Characteristics:

K_D (binding affinity):	0.43 nM
B_{max} (receptor number):	30.9 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source:	Rat cortical membranes
Radioligand:	[^{3}H]Ketanserin (60-90 Ci/mmol)
	Final ligand concentration - [1.0nM]
Non-specific Determinant:	Methysergide - [100 µM]
Reference Compound:	Methysergide
Positive Control:	Methysergide
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.6) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the $5HT_2$ binding site.

Literature Reference:

Leysen, J. E., Niemegeers, C. J., Van Nueten, J. M. and Laduron, P. M. [^{3}H]Ketanserin: A Selective Tritiated Ligand for Serotonin$_2$ Receptor Binding Sites. *Mol. Pharmacol.* **21:** 301-314 (1982) with modifications.

Martin, G.R. and Humphrey, P.P.A. Classification Review: Receptors for 5-HT: Current Perspectives on Classification and Nomenclature. *Neuropharmacol.* **33(3/4):** 261-273 (1994).

SEROTONIN, 5HT$_{1D}$ (HUMAN)
BINDING ASSAY

Reference Compounds	Ki (nM)
■ 5CT	0.7
○ 5HT	10.0
▲ 8OH-DPAT	921.0
✕ MDL72222	10,000
◇ Mesulergine	50,000

Assay Characteristics:

K_D (binding affinity):	2.5 nM
B_{max} (receptor number):	2.1 fmol/mg tissue

Materials and Methods:

Receptor Source:	Human cerebral cortical membranes
Radioligand:	[^{3}H] 5-Carboxamidotryptamine (20-70 Ci/mmol)
	Final ligand concentration - [0.75 nM]
Non-specific Determinant:	5- Carboxamidotryptamine (5-CT) - [1.0 μM]
Reference Compound:	5- Carboxamidotryptamine (5-CT)
Positive Control:	5- Carboxamidotryptamine (5-CT)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 4 mM CaCl$_2$, 100 nM 8-OH-DPAT, 100 nM Mesulergine, 10 uM Pargyline and 0.1% ascorbic acid at 25^{0}C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned 5HT$_{1D}$ binding site.

Literature Reference:	Waeber C., Schoeffter, Palacios, J.M. and Hoyer, D. Molecular Pharmacology of the 5-HT$_{1D}$ Recognition Sites: Radioligand Binding Studies in Human, Pig, and Calf Brain Membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **337**: 595-601 (1988) with modifications.

SEROTONIN, 5HT$_{2C}$
BINDING ASSAY
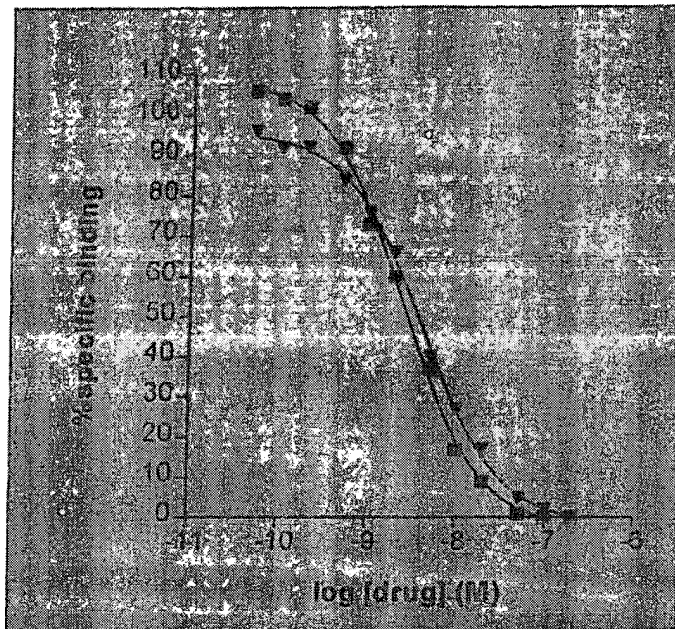
Reference Compounds		Ki (nM)
□	Mianserin	1.7
▼	Mesulergine	2.1
	Methysergide	3.2
	Ketanserin	27.5

Assay Characteristics:

K$_D$ (binding affinity): 1.1 nM
B$_{max}$ (receptor number): 300 fmol/mg protein

Materials and Methods:

Receptor Source: Pig choroid plexus membranes
Radioligand: [^{3}H] Mesulgerine (50-60 Ci/mmol)
  Final ligand concentration - [1.0 nM]
Non-specific Determinant: Serotonin - [100 μM]
Reference Compound: Mianserin
Positive Control: Mianserin
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 4 mM CaCl$_2$ and 0.1% ascorbic acid at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT$_{2C}$ binding site.

Literature Reference: A. Pazos, D. Hoyer, and J. Palacios. The Binding of Serotonergic Ligands to the Porcine Choroid Plexus: Characterization of a New Type of Serotonin Recognition Site. *Eur. Jrnl. Pharmacol.* **106**: 539-546 (1985) with modifications.

Hoyer, D., Engel, G., et al. Molecular Pharmacology of 5HT$_1$ and 5-HT$_2$ Recognition Sites in Rat and Pig Brain Membranes: Radioligand Binding Studies with [^{3}H]-5HT, [^{3}H]-8-OH-DPAT, [^{125}I]-Iodocyanopindolol, [^{3}H]-Mesulergine and [^{3}H]-Ketanserin. *Eur. Jrnl. Pharmacol.* **118**: 13-23 (1985) with modifications.

SEROTONIN, 5HT$_{2A}$ (HUMAN)
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Ketanserin	4.0
○ Mesulergine	701.0
▼ 5-HT	1540.0
× 5-CT	7630.0
❑ 8OH-DPAT	>10,000
● MDL 72222	26,350

Assay Characteristics:

K$_D$ (binding affinity): 2 nM
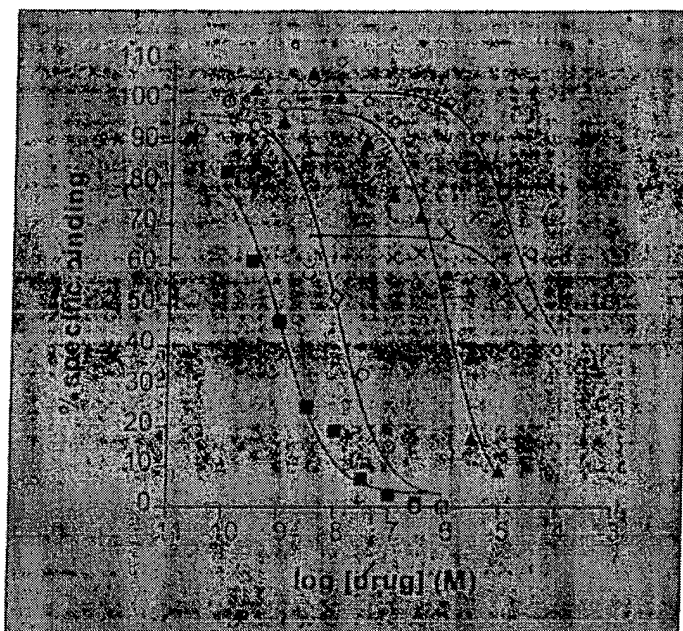B$_{max}$ (receptor number): 10 fmol/mg tissue

Materials and Methods:

Receptor Source: Human Cortex
Radioligand: [^{3}H] Ketanserin (60-90 Ci/mmol)
  Final ligand concentration - [1.0 nM]
Non-specific Determinant: Ketanserin - [1.0 μM]
Reference Compound: Ketanserin
Positive Control: Ketanserin
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.5) at room temperature for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT$_{2A}$ binding site.

Literature Reference: Leysen, J. E., Niemegeers, C. J., Van Nueten, J. M. and Laduron, P. M. [^{3}H]Ketanserin: A Selective Tritiated Ligand for Serotonin$_2$ Receptor Binding Sites. *Mol. Pharmacol.* **21**: 301-314 (1982) with modifications.

Martin, G.R. and Humphrey, P.P.A. Classification Review: Receptors for 5-HT: Current Perspectives on Classification and Nomenclature. *Neuropharmacol.* **33(3/4)**: 261-273 (1994).

SEROTONIN, 5HT$_4$
BINDING ASSAY
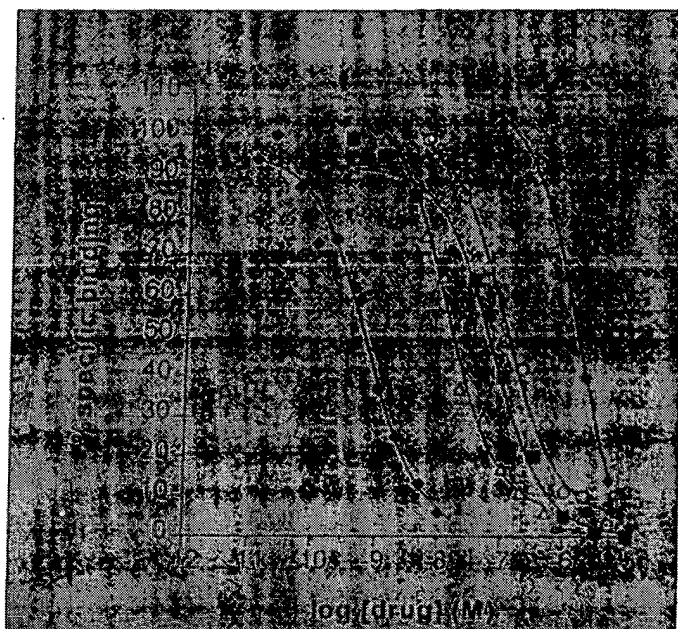
Reference Compounds		Ki (nM)
◆	GR113808	0.6
△	BIMU	11.5
■	Serotonin (5HT)	14.5
○	Renzapride	101.0
✳	Methiothepin	715.0

Assay Characteristics:

K$_D$ (binding affinity):	0.2 nM
B$_{max}$ (receptor number):	25 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Guinea pig striatal membranes

Radioligand: [^{3}H]-113808 (30-70 Ci/mmol)

Final ligand concentration - [0.2 nM]

Non-specific Determinant: Serotonin (5-HT) - [30 μM]

Reference Compound: Serotonin (5-HT)

Positive Control: Serotonin (5-HT)

Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.4) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT$_4$ binding site.

Literature Reference:

Grossman, Kilpatrick, C., et al. Development of a Radioligand Binding Assay for 5HT$_4$ Receptors in Guinea Pig and Rat Brain. *Brit. Jrnl. Phamacol.* **109**: 618-624 (1993).

SEROTONIN, 5HT₃
BINDING ASSAY
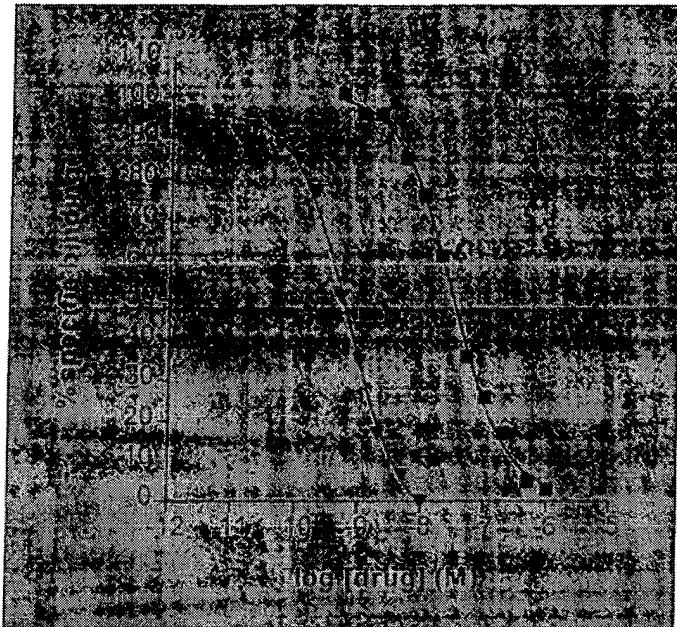
Reference Compounds	Ki (nM)
▼ ICS 205,930	0.2
Quipazine	1.5
■ MDL-72222	7.0
Serotonin	18.7
Metoclopramide	191.0

Assay Characteristics:

K_D (binding affinity):	0.35 nM
B_{max} (receptor number):	233 fmol/10^6 cells

Materials and Methods:

Receptor Source:	N1E-115 cells
Radioligand:	[³H]-GR65630 (30-70 Ci/mmol)
	Final ligand concentration - [0.35 nM]
Non-specific Determinant:	MDL-72222 - [1.0 µM]
Reference Compound:	MDL-72222
Positive Control:	MDL-72222
Incubation Conditions:	Reactions are carried out in 20 mM HEPES (pH 7.4) containing 150 mM NaCl at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5HT₃ binding site.

Literature Reference:

Lummis, S.C.R., Kilpatrick, G.J.. Characterization of 5HT₃ Receptors in Intact N1E-115 Neuroblastoma Cells. *Eur. Jrnl. Pharmacol.* **189**: 223-227 (1990) with modifications.

Hoyer, D. and Neijt, H.C. Identification of Serotonin 5-HT₃ Recognition Sites in Membranes of N1E-115 Neuroblastoma Cells by Radioligand Binding. *Mol. Pharmacol.* **33**: 303 (1988).

Tyers, M.B. 5-HT₃ Receptors and the Therapeutic Potential of 5-HT₃ Receptor Antagonists. *Therapie.* **46**: 431-435 (1991).

SEROTONIN, 5HT$_6$ (HUMAN RECOMBINANT) BINDING ASSAY
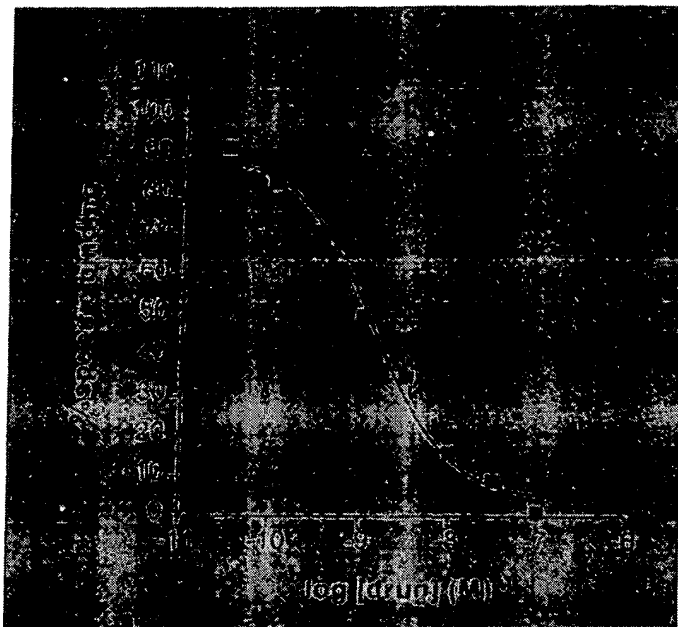
Reference Compounds	Ki (nM)
■ Methiothepin mesylate	5.2

Assay Characteristics:

K$_D$ (binding affinity):	1.5 nM
B$_{max}$ (receptor number):	3000 fmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in HEK293 cells
Radioligand:	[^{3}H]LSD (60-80 Ci/mmol)
	Final ligand concentration - [1.5 nM]
Non-specific Determinant:	Methiothepin - [0.1 μM]
Reference Compound:	Methiothepin
Positive Control:	Methiothepin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl$_2$, 0.5 mM EDTA for 60 minutes at 37°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned serotonin - 5HT$_6$ binding site.

Literature Reference: Monsma, F.J. Jr., et al., Molecular Cloning and Expression of Novel Serotonin Receptor with High Affinity for Tricyclic Psychotropic Drugs. *Mol. Pharmacol.* (43): 320-327 (1993).

SEROTONIN, 5HT$_{5A}$ (HUMAN RECOMBINANT)
BINDING ASSAY
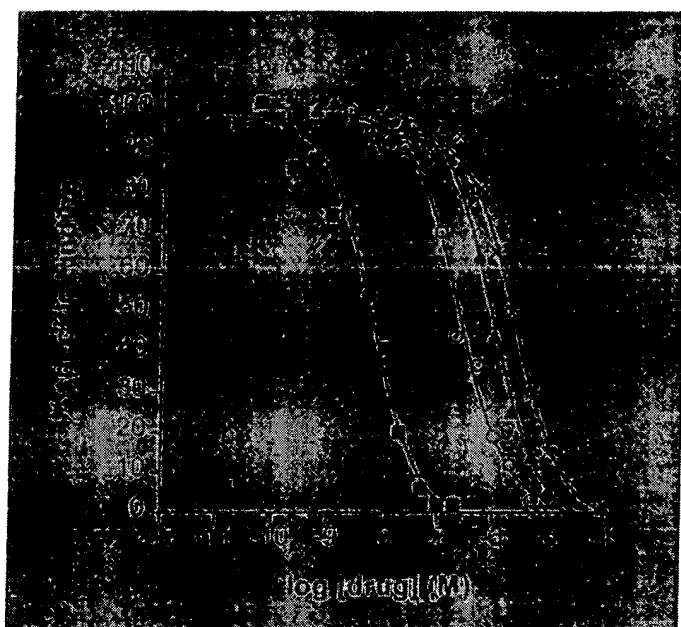
Reference Compound		Ki (nM)
■	Methiothepin	5.7
○	Metergoline	269.0
▲	Mianserin	409.9
×	Clozapine	1818.5

Assay Characteristics:

K_D (binding affinity):	1.5 nM
B_{max} (receptor number):	2 - 4 pmol/mg protein

Materials and Methods:

|	
Receptor Source:	Human Recombinant expressed in HEK 293 cells
Radioligand:	[^{3}H] LSD (60-87 Ci/mmol)
	Final ligand concentration - [1.0 nM]
Non-specific Determinant:	Methiothepin - [1.0 μM]
Reference Compound:	Methiothepin
Positive Control:	Methiothepin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgSO$_4$ and 0.5 mM EDTA at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the cloned 5HT$_{5A}$ binding site.

Literature Reference: Rees, S., et al. *FEBS Letters*, **355**: 242-246 (1994) with modifications

SEROTONIN, NON-SELECTIVE
BINDING ASSAY
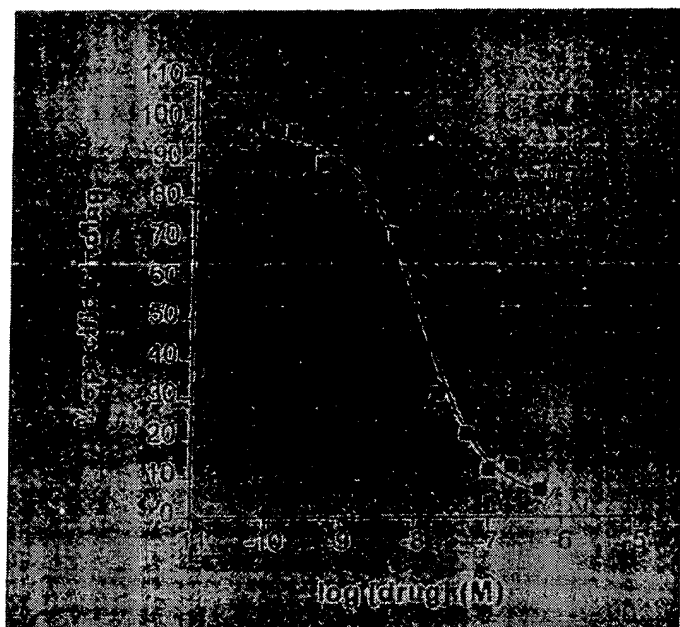
Reference Compounds	Ki (nM)
■ Methysergide	5.7
Spiroperidol	18.0
Mianserin	33.0
5-Methoxytryptamine	210.0

Assay Characteristics:

K_D (binding affinity):   5.1 nM
B_{max} (receptor number):   23 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat cortical membranes
Radioligand: [^{3}H]Lysergic acid diethylamide (60-70 Ci/mmol)
      Final ligand concentration - [5.0 nM]
Non-specific Determinant: Methysergide -[10.0 uM]
Reference Compound: Methysergide
Positive Control: Methysergide
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 4 mM $CaCl_2$, 0.1 mM pargyline and 0.1% ascorbic acid at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the serotonin binding site.

Literature Reference: Peroutka, S.J. and Snyder, S.H. Multiple Serotonin Receptors: Differential Binding of [^{3}H]5-Hydroxytryptamine, [^{3}H]Lysergic Acid Diethylamide and [^{3}H]Spiroperidol. *Mol. Pharmacol.* **16**: 687-699 (1979) with modifications.

Peroutka S.J. and Snyder, S.H. Two Distinct Serotonin Receptors: Regional Variations in Receptor Binding in Mammalian Brain. *Brain Research.* **208**: 339-347 (1981).

SEROTONIN, 5HT$_7$ (HUMAN RECOMBINANT)
BINDING ASSAY
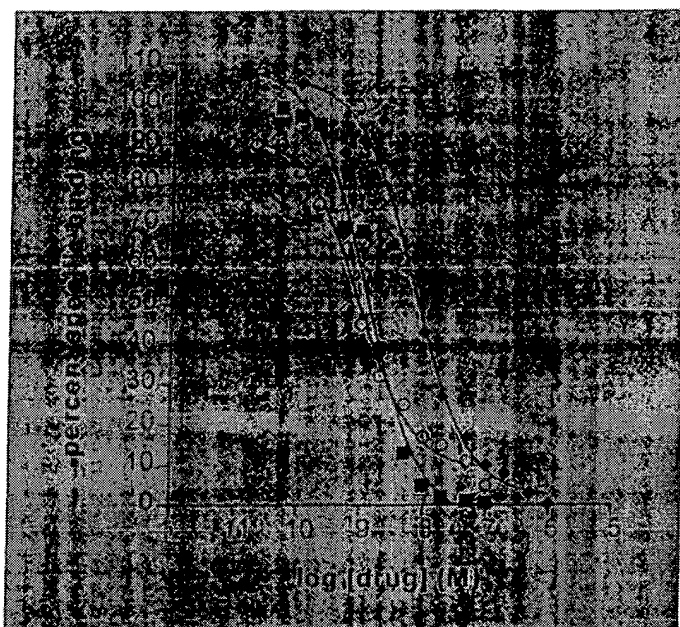
Reference Compounds	Ki (nM)
○ 5-CT	0.5
■ Methiothepin	0.7
◆ 5-HT	4.0

Assay Characteristics:

K_D (binding affinity):	4.1 nM
B_{max} (receptor number):	7 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in HE-293 cells
Radioligand:	[^{3}H]LSD (60-80 Ci/mmol)
	Final ligand concentration - [3.0 nM]
Non-specific Determinant:	Methiothepin - [0.1 μM]
Reference Compound:	Methiothepin
Positive Control:	Methiothepin
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl$_2$, 0.5 mM EDTA for 60 minutes at 37°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound(s) with the cloned serotonin - 5HT$_7$ binding site.

Literature Reference: Shen, Y. et al., Molecular Cloning and Expression of a 5-hydroxytryptamine$_7$ Serotonin Receptor Subtype., Jrnl. Biol. Chem. **(268)**: 18200-18204 (1993).

GenBank Accession Number: L21195

SEROTONIN TRANSPORTER (HUMAN)
BINDING ASSAY
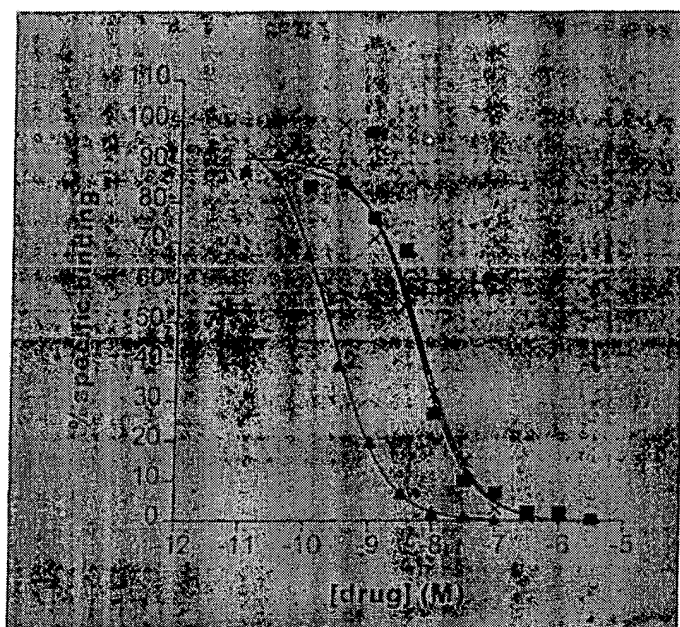
Reference Compound		Ki (nM)
▲	Clomipramine	0.2
✕	Citalopram	3.0
■	Imipramine	4.0

Assay Characteristics:

K_D (binding affinity):	2.5 nM
B_{max} (receptor number):	425 fmol/mg protein

Materials and Methods:

Receptor Source:	Human platelet membranes
Radioligand:	[³H]Citalopram (70-87 Ci/mmol)
	Final ligand concentration - [0.7 nM]
Non-specific Determinant:	Clomipramine - [1.0 μM]
Reference Compound:	Imipramine
Positive Control:	Imipramine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4), containing 120 mM NaCl and 5 mM KCl at 25⁰C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined using liquid scintillation spectrometry and compared to control values in order to ascertain any interactions of test compound with the serotonin transporter binding site.

Literature Reference:

D'Amato, R.J., Largent, B.L., Snowman, A.M., and Snyder, S.H. Selective Labeling of Serotonin Uptake Sites in Rat Brain by [³H]Citalopram Contrasted to Labeling of Multiple Sites by [³H]Imipramine. *Jrnl. Pharmacol. & Exp. Ther.* **242:** 364-371 (1987) with modifications.

Brown, N.L., Sirugue, O. and Worcel, M. The Effects of Some Slow Channel Blocking Drugs on High Affinity Serotonin Uptake by Rat Brain Synaptosomes. *Eur. Jrnl. Pharmac.* **123:** 161-165 (1986).

SEROTONIN TRANSPORTER
BINDING ASSAY

Reference Compounds	Ki (nM)
Paroxetine	0.1
Fluoxetine	1.4
▼ Clomipramine	2.8
■ Imipramine	40.9
Serotonin	55.6
Zimelidine	68.3

Assay Characteristics:

K_D (binding affinity):   1.7 nM

B_{max} (receptor number):   33.1 fmol/mg protein

Materials and Methods:

|	
Receptor Source.	Rat forebrain membranes
Radioligand:	[^{3}H]Citalopram (70-87 Ci/mmol)
	Final ligand concentration - [0.7 nM]
Non-specific Determinant:	Clomipramine - [10 μM]
Reference Compound:	Imipramine
Positive Control:	Imipramine
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4), containing 120 mM NaCl and 5 mM KCl at 25^{0}C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined using liquid scintillation spectrometry and compared to control values in order to ascertain any interactions of test compound with the serotonin transporter binding site.

Literature Reference: D'Amato, R.J., Largent, B.L., Snowman, A.M., and Snyder, S.H. Selective Labeling of Serotonin Uptake Sites in Rat Brain by [^{3}H]Citalopram Contrasted to Labeling of Multiple Sites by [^{3}H]Imipramine. *Jrnl. Pharmacol. & Exp. Ther.* **242:** 364-371 (1987) with modifications.

Brown, N.L., Sirugue, O. and Worcel, M. The Effects of Some Slow Channel Blocking Drugs on High Affinity Serotonin Uptake by Rat Brain Synaptosomes. *Eur. Jrnl. Pharmac.* **123:** 161-165 (1986).

SIGMA₁
BINDING ASSAY
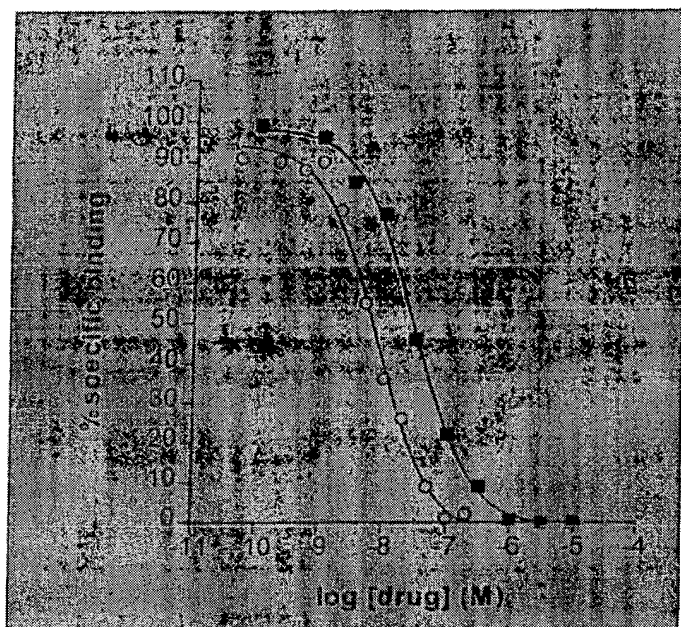
Reference Compounds	Ki (nM)
○ Haloperidol	4.7
■ 3(+)-PPP	20.9

Assay Characteristics:

K_D (binding affinity):	11 nM
B_{max} (receptor number):	27 pmol/mg tissue

Materials and Methods:

Receptor Source:	Guinea pig brain membranes
Radioligand:	[³H]-(+)-Pentazocine (30-60 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	(+)-3-PPP - [10.0 μM]
Reference Compound:	(+)-3-PPP
Positive Control:	(+)-3-PPP
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 8.0) at 25°C for 120 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the sigma₁ binding site.

Literature Reference:
Chaki, S., Tanaka, M., Muramatsu, M., and Otomo, S., NE-100, A Novel Potent σ Ligand, Preferentially Binds to σ₁ Binding Sites in Guinea Pig Brain. *Euro. J. Pharmacol.* 251: R1-R2 (1994).

Serotonin Transport (Human)
Assay
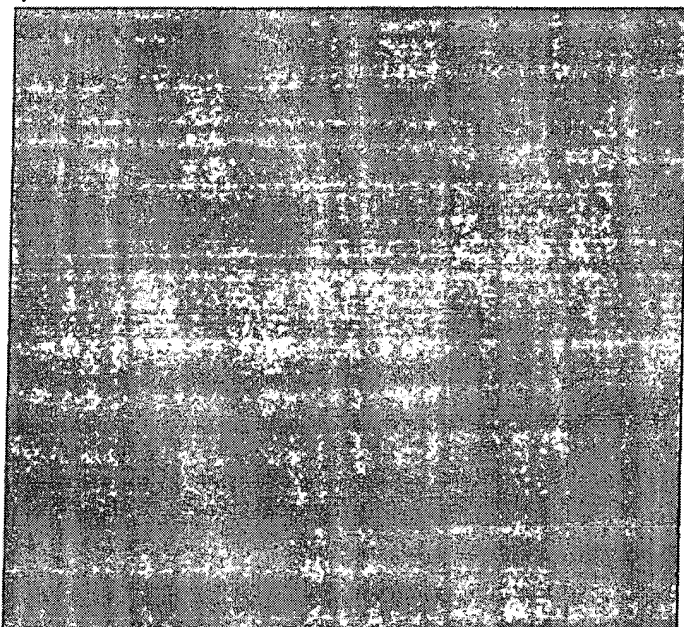
SIGMA, NON-SELECTIVE
BINDING ASSAY
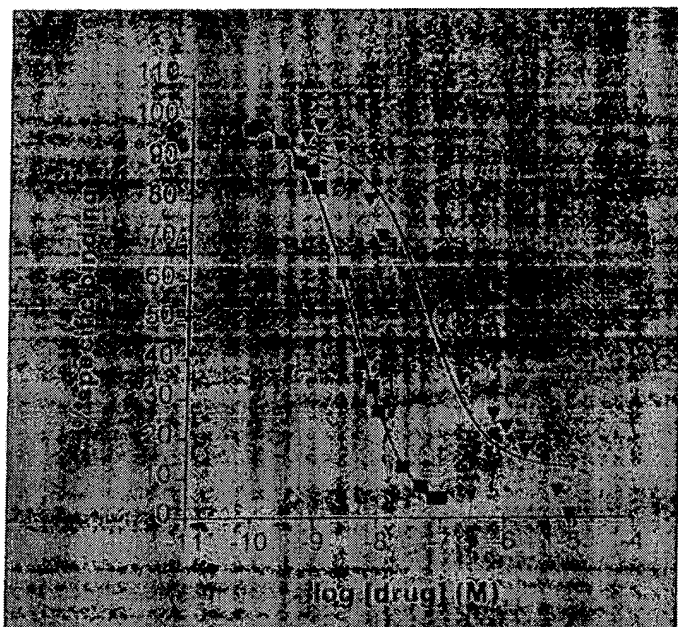
Reference Compounds	Ki (nM)
■ Haloperidol	7.0
DTG	31.0
▼ (+) Pentazocine	59.6
(+)-3-PPP	124.6
(+)-SKF-10047	1,263.0

Assay Characteristics:

K_D (binding affinity):	39.2 nM
B_{max} (receptor number):	19.7 fmol/mg protein

Materials and Methods:

Receptor Source:	Guinea pig brain membranes
Radioligand:	DTG [5-^{3}H](1,3-Di-O-2-Tolylguanidine-DI-[p-Ring-^{3}H]) (30-60 Ci/mmol) Final ligand concentration - [4.0 nM]
Non-specific Determinant:	Haloperidol - [1.0 µM]
Reference Compound:	Haloperidol
Positive Control:	Haloperidol
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the sigma binding site.

Literature Reference:

Weber, E., Sonders, M., Quarum, M., et al. [^{3}H]Di(2-tolyl)quanidine: A Selective Ligand that Labels Sigma-Type Receptors for Psychotomimetic Opiates and Antipsychotic Drugs. *Proc. Nat'l Acad. Sci.* **83**: 8784-8788 (1986) with modifications.

Karbon, E.W., Naper, K., and Pontecorvo, M.J. [^{3}H]DTG and [^{3}H]-3-PPP Label Pharmacologically Distinct σ Binding Sites in Guinea Pig Brain. *Eur. Jrnl. Pharmac.* **193**: 21-27 (1991).

SIGMA$_2$
BINDING ASSAY
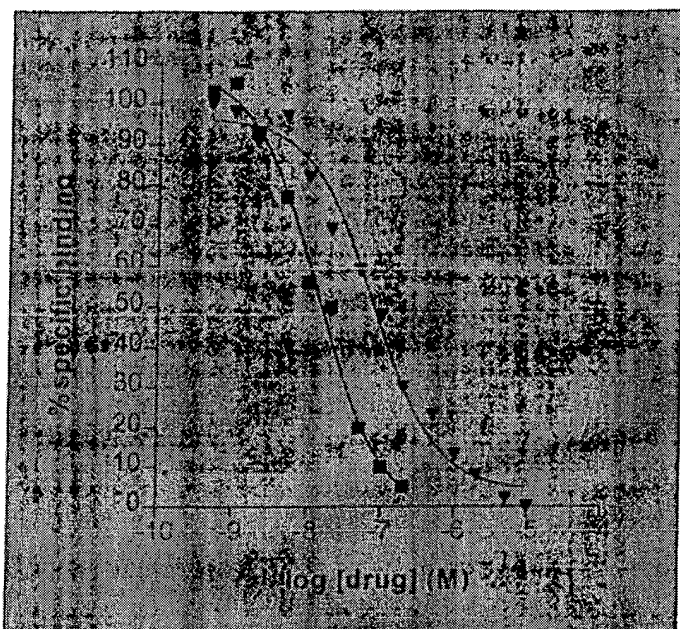
Reference Compounds	Ki (nM)
■ Haloperidol	13.8
▼ DTG	72.0

Assay Characteristics:

|	
K$_D$ (binding affinity):	39.2 nM
B$_{max}$ (receptor number):	17 pmol/mg tissue

Materials and Methods:

Receptor Source:	Guinea pig brain membranes
Radioligand:	[^{3}H]DTG (+ 100nM (+)-Pentazocine) (30-60 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	Haloperidol - [1.0 μM]
Reference Compound:	Haloperidol
Positive Control:	Haloperidol
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) at 25°C for 90 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the sigma$_2$ binding site.

Literature Reference:	Chaki, S., Tanaka, M., Muramatsu, M., and Otomo, S., NE-100, A Novel Ootent σ Ligand, Preferentially Binds to σ$_1$ Binding Sites in Guinea Pig Brain. *Euro. J. Pharmacol.* 251: R1-R2 (1994) with modifications.

SODIUM CHANNEL, SITE 2
BINDING ASSAY
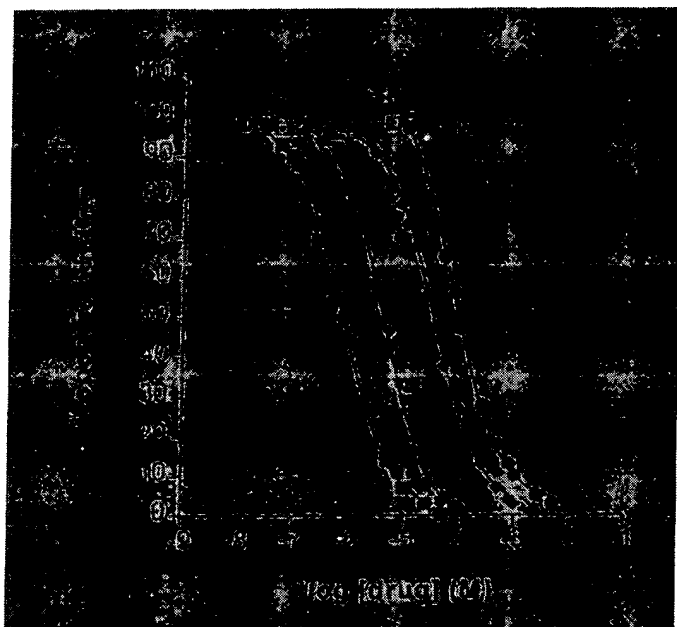
Reference Compounds		Ki (μM)
	Dibucaine	0.9
■	Aconitine	1.0
	Tetracaine	2.8
▼	Veratridine	3.6
	Bupivacaine	3.8
♦	Lidocaine	29.9
●	Procaine	92.3
	Procainamide	189.0
☐	Tetrodotoxin	>100.0

Assay Characteristics:

K_D (binding affinity):   32 nM
B_{max} (receptor number):   52 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat forebrain membranes
Radioligand: [³H]Batrachotoxin (30-60 Ci/mmol)
                          Final ligand concentration - [2.0 nM]
Non-specific Determinant: Aconitine - [0.1 mM]
Reference Compound: Aconitine
Positive Control: Veratridine
Incubation Conditions: Reactions are carried out in 50 nM HEPES (pH 7.4) containing 130 mM choline chloride at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the sodium channel, site 2 binding site.

Literature Reference: Creveling, C. R. Batrachotoxin-Induced Depolarization and [³H]Batrachotoxin - A 20a - Benzoate Binding in a Vesicular Preparation from Guinea Pig Cerebral Cortex. *Mol. Pharmacol.* **23:** 350-358 (1983) with modifications.

                          Trainer, V.L., Moraeu, E., et al. Neurotoxin Binding and Allosteric Modulation at Receptor Sites 2 and 5 on Purified and Reconstituted Rat Brain Sodium Channels. *Jrnl. Biol. Chem.* **268(23):** 17114-17119 (1993).

SODIUM CHANNEL, SITE 1
BINDING ASSAY
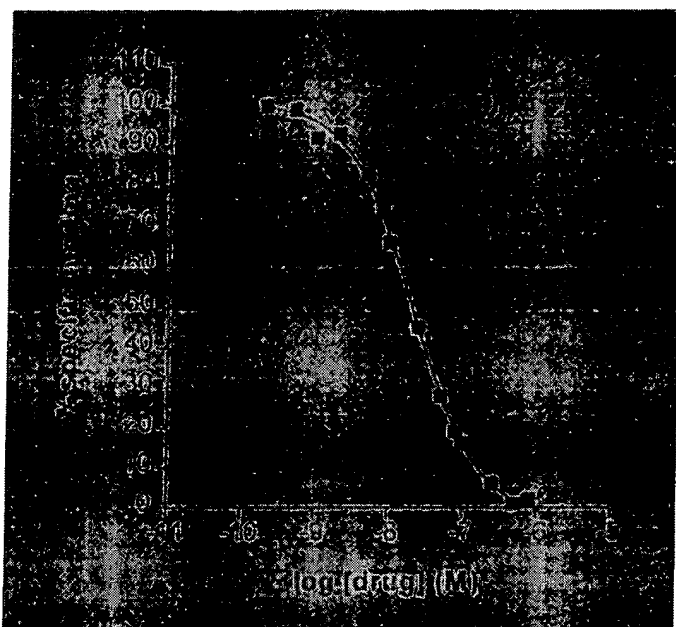
Reference Compounds	Ki (nM)
■ Tetrodotoxin	12.0
Aconitine	> 100,000
Lidocaine	> 100,000
Procaine	> 100,000

Assay Characteristics:

K_D (binding affinity):	2.2 nM
B_{max} (receptor number):	2.4 pmol/mg protein

Materials and Methods:

Receptor Source:	Rat forebrain membranes
Radioligand:	[^{3}H]Saxitoxin (20-40 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	Tetrodotoxin - [10.0 μM]
Reference Compound:	Tetrodotoxin
Positive Control:	Tetrodotoxin
Incubation Conditions:	Reactions are carried out in 130 mM choline chloride (pH 7.4) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration of the reaction contents onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the sodium channel, site 1 binding site.

Literature Reference:

Ohizumi, Yasushi. Specific Inhibition of [^{3}H]Saxitoxin Binding to Skeletal Muscle Sodium Channels by Geopraphutoxin II, a Polypeptide Channel Blocker. *Jrnl. Biol. Chem.* **261**: 6149-6152 (1986) with modifications.

Rubin, J.G. and Soderlund, D.M. Binding of [^{3}H]Batrachotoxin A-20-Alpha-Benzoate and [^{3}H]Saxitoxin to Receptor Sites Associated with Sodium Channels in Trout Brain Synaptoneurosomes. *Comp. Biochem. Physiol.* **105(2)**: 231-238 (1993).

TESTOSTERONE
BINDING ASSAY
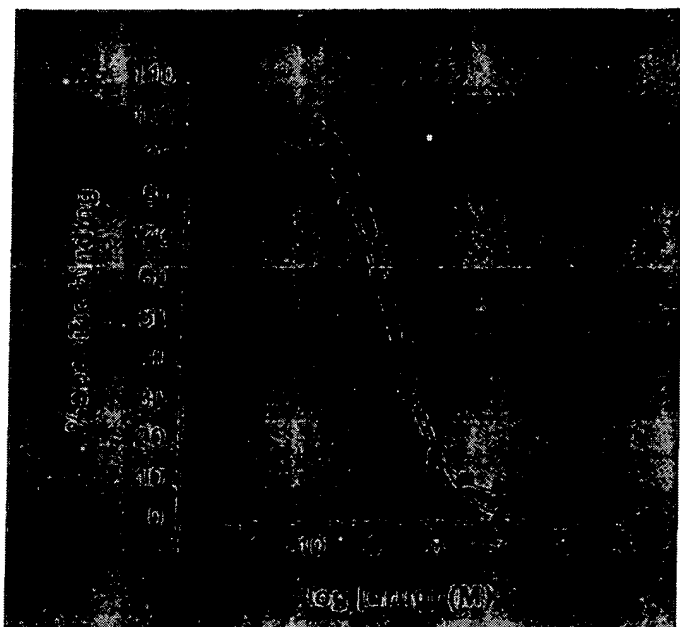
Reference Compounds	Ki (nM)
Mibolerone	0.5
■ Methyltrienolone (R1881)	1.4
▲ Dihydrotestosterone	2.3
17-β-Estradiol	93.9
Progesterone	124.0

Assay Characteristics:

K_D (binding affinity):	4.0 nM
B_{max} (receptor number):	125 fmol/mg tissue (wet weight)

## Materials and Methods:

Receptor Source:	Rat prostate cytosol
Radioligand:	[^{3}H]Methyltrienolone (R1881) (70-87 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specific Determinant:	Methyltrienolone (R1881) - [10 μM]
Reference Compound:	Methyltrienolone (R1881)
Positive Control:	Methyltrienolone (R1881)
Incubation Conditions:	Reactions are carried out in 25 mM HEPES buffer (pH 7.4) containing 1.0 mM EDTA, 10 mM sodium molybdate, 10% glycerol, 0.2 mM leupeptin, and 0.5 mM PMSF at 0-4°C for 18 hours. The reaction is terminated by the addition of dextran coated charcoal and incubated for 10 minutes at 0-4°C. The reaction mixtures are centrifuged and the radioactivity bound in the supernatant is compared to control values in order to ascertain any interactions of test compound with the testosterone binding site.

Literature Reference:

Traish, A.M., Muller, R.E., and Wotiz, H.H. Binding of 7α, 17α-Dimethyl-19-Nortestosterone (Mibolerone) to Androgen and Progesterone Receptors in Human and Animal Tissue. *Endocrinology.* **118:** 1327-1333 (1986) with modifications.

Zava, D.T., Landrum, B., et al. Androgen Receptor Assay with [^{3}H]Methyltrienolone (R1881) in the Presence of Progesterone Receptors. *Endocrinology.* **104:** 1007-1012 (1979).

SOMATOSTATIN
BINDING ASSAY
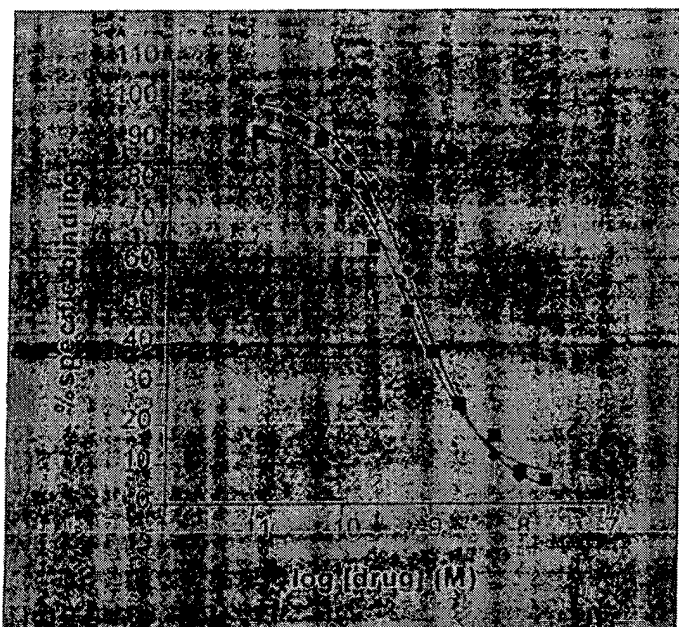
Reference Compounds	Ki (nM)
■ Somatostatin	0.1
[Tyr⁰, D-Trp⁸]Somatostatin	0.1
Somatostatin 28	0.2
◆ [Try¹¹]Somatostatin	0.6

Assay Characteristics:

K_D (binding affinity):	1.0 nM
B_{max} (receptor number):	20 fmol/mg tissue (wet weight)

Materials and Methods:

Receptor Source: Rat forebrain membranes

Radioligand: [^{125}I]Somatostatin (2000 Ci/mmol)
Final ligand concentration - [0.08 nM]

Non-specific Determinant: Somatostatin - [1.0 uM]

Reference Compound: Somatostatin

Positive Control: Somatostatin

Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.5) containing 500 kiu/ml aprotinin, 0.02 mg/ml bacitracin, 0.1% BSA and 5 mM MgCl$_2$ at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the somatostatin binding site.

Literature Reference: Raynor, K., Reisine, T. Analogs of Somatostatin Selectivity Label Distinct Subtypes of Somatostatin Receptors in Rat Brain. *Jrnl. Pharmacol. Exp. Ther.* **251**: 510-517 (1989) with modifications.

Czernik, A.J. and Petrack, B. Somatostatin Receptor Binding in Rat Cerebral Cortex. Characterization using a Nonreducible Somatostatin Analog. *Jrnl. Biol. Chem.* **258(9)**: 5525-5530 (1983).

Reubi, J-C., Perrin, M.H., Rivier, J.E. and Vale, W. High Affinity Binding Sites for a Somatostatin-28 Analog in Rat Brain. *Life Sci.* **28**: 2191-2198 (1981).

THYROTROPIN RELEASING HORMONE (TRH)
BINDING ASSAY
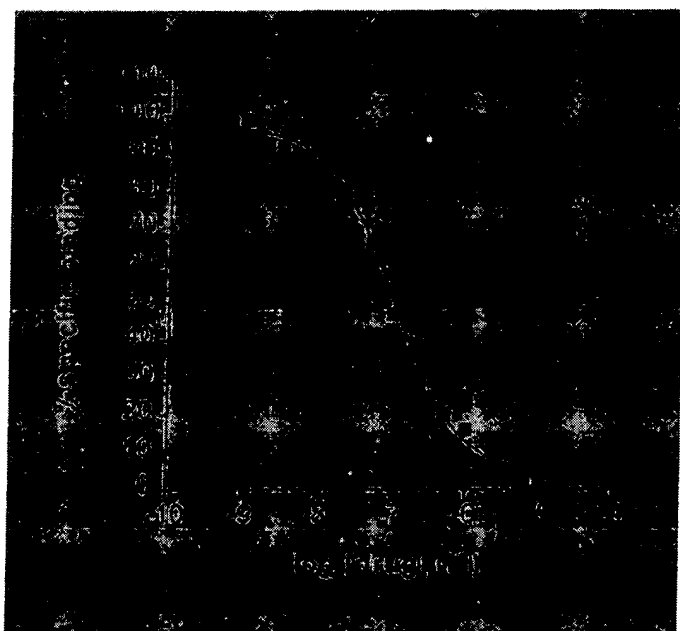
Reference Compounds	Ki (nM)
[3MeHis²]-TRH	2.5
■ TRH	42.3

Assay Characteristics:

K_D (binding affinity):   2.3 nM
B_{max} (receptor number):   34.0 fmol/mg protein

Materials and Methods:

Receptor Source: Rat forebrain membranes
Radioligand: [³H]-(3-MeHis²)Thyrotropin releasing hormone (40.0-70.0 Ci/mmol)
                              Final ligand concentration - [2.0 nM]
Non-specific Determinant: Thyrotropin releasing hormone (TRH) - [10.0 uM]
Reference Compound: Thyrotropin releasing hormone (TRH)
Positive Control: Thyrotropin releasing hormone (TRH)
Incubation Conditions: Reactions are carried out in cold 20 mM Na₂HPO₄ (pH 7.4) at 4⁰C for 3-4 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the TRH binding site.

Literature Reference:

Simasko, S. and Horita, A. Characterization and Distribution of [³H]-(3MeHis²) Thyrotropin Releasing Hormone Receptors in Rat Brain. *Life Sciences.* **30(21):** 1793-1799 (1982) with modifications.

Burt, D.R. and Snyder, S.H. TRH: Apparent Receptor Binding in Rat Brain Membranes. *Brain Res.* **93:** 309-328 (1975).

Taylor, R.L. and Burt, D.R. Preparation of [³H]3-Me-His²-TRH as an Improved Ligand for TRH Receptors. *Neuroendocrin.* **32:** 310-316 (1981).

THROMBOXANE A_2
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Pinane-thromboxane	149.0

Assay Characteristics:

K_D (binding affinity):	2.0 nM
B_{max} (receptor number):	1800 fmol/mg protein

Materials and Methods:

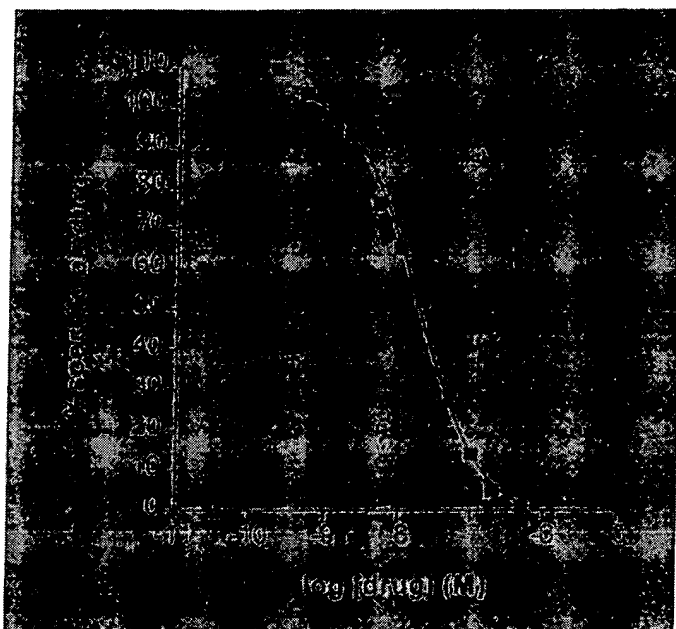|	
Receptor Source:	Human platelets
Radioligand:	[^{3}H]SQ 29,548 (30-60 Ci/mmol)
	Final ligand concentration - [2.0 nM]
Non-specific Determinant:	Pinane-thromboxane - [10 μM]
Reference Compound:	Pinane-thromboxane
Positive Control:	Pinane-thromboxane
Incubation Conditions:	Reactions are carried out in 25 mM TRIS-HCl (pH 7.4) containing 138 mM NaCl, 5 mM KCl, 5 mM MgCl$_2$, 5.5 mM dextrose, and 2 mM EDTA at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the thromboxane A_2 binding site.

Literature Reference:

Hedberg, A., et al. Characterization of [^{3}H]SQ 29,548 as a High Affinity Radioligand Binding to Thromboxane A_2 - Receptors in Human Platelets. *Jrnl. Pharmacol. Exp. Ther.* **245**: 786-792 (1988) with modifications.

Armstrong, R. A., Jones, R. L., et al. Ligand Binding to Thromboxane Receptors on Human Platelets: Correlation with Biological Activity. *Brit. Jrnl. Pharmac.* **79**: 953-964 (1983).

VASOACTIVE INTESTINAL PEPTIDE, PACAP SPLICE VARIANT 1 (HUMAN RECOMBINANT) BINDING ASSAY
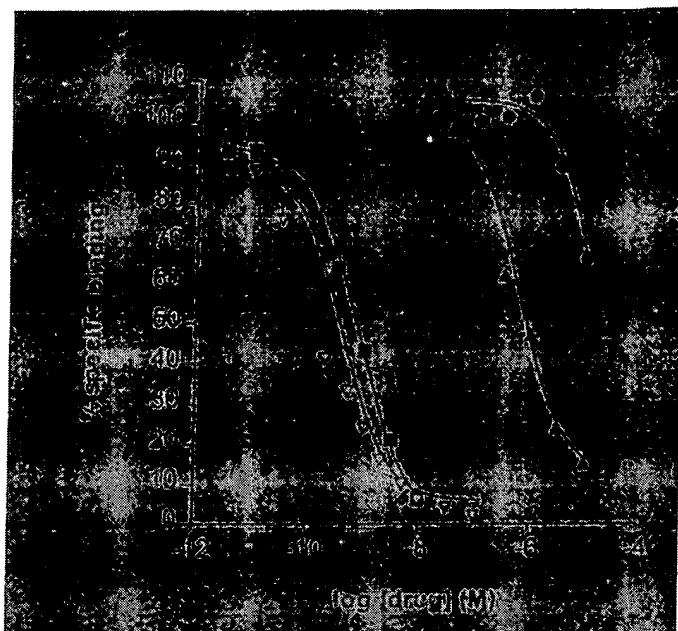
Reference Compounds	Ki (nM)
▽ PACAP$_{1-38}$	0.18
■ PACAP$_{1-27}$	0.58
▲ VIP	471.0
● VIP$_{10-28}$	>10,000

Assay Characteristics:

K_D (binding affinity):	0.7 nM
B_{max} (receptor number):	6.9 pmol/mg protein

Materials and Methods:

Receptor Source:	Human recombinant expressed in NIH-3T3 cells
Radioligand:	[^{125}I]PACAP (2000 Ci/mmol)
	Final ligand concentration - [0.07 nM]
Non-specific Determinant:	PACAP$_{1-27}$ - [0.1 uM]
Reference Compound:	PACAP$_{1-27}$
Positive Control:	PACAP$_{1-27}$
Incubation Conditions:	Reactions are carried out in 20 mM TRIS-HCl (pH 7.4) containing 5 mM MgCl$_2$, 0.1% BSA and 0.1 mg/ml bacitracin at 25°C for 90 minutes. The reaction is terminated by filtration and amount of radioactivity is determined and compared to control values in order to ascertain any interactions of test compound with the PACAP binding site.

Literature Reference: Hosoya, M. et al. Molecular Cloning and Functional Expression of Rat cDNAs Encoding the Receptor for Pituitary Adenylate Cyclase Activating Polypeptide (PACAP). *Biochem. and Biophys. Research Commun.* **194(1):** 133-143 (1993) with modifications.

VASOACTIVE INTESTINAL PEPTIDE (VIP), NON-SELECTVE BINDING ASSAY
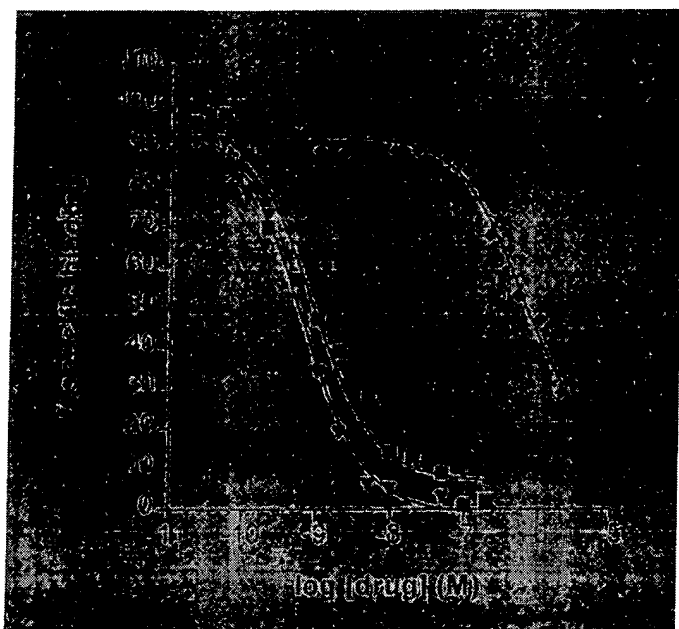
Reference Compounds	Ki (nM)
▼ PACAP$_{(1-27)}$ Amide	0.6
■ VIP (rat/human/porcine)	0.8
[Ac-Tyr^1, D-Phe^2]-GRF	461.5
◆ VIP$_{(10-28)}$	536.0
pCL-VIP	2,224.0
VIP $_{(1-2)}$	10,000.0

Assay Characteristics:

K_D (binding affinity): 1.0 nM
B_{max} (receptor number): 11.0 fmol/mg tissue (wet weight)
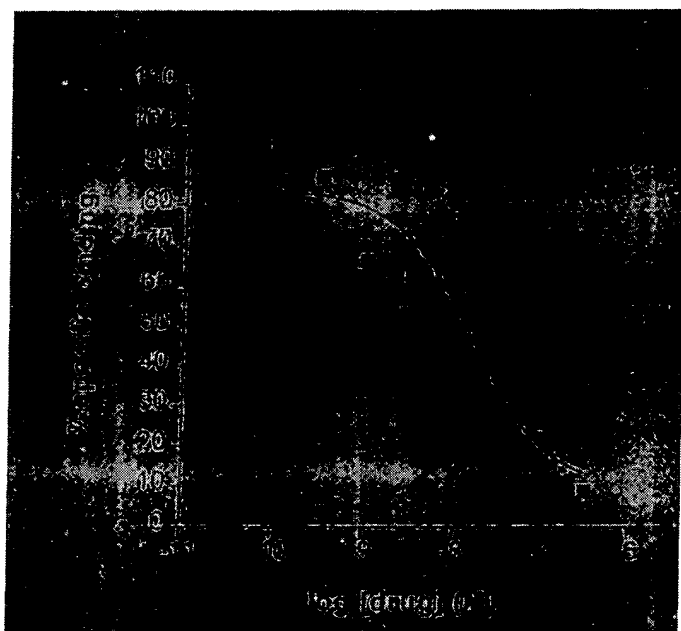
Materials and Methods:

Receptor Source: Rat forebrain membranes
Radioligand: [^{125}I]Vasoactive intestinal peptide (2200 Ci/mmol)
Final ligand concentration - [0.05 nM]
Non-specific Determinant: Vasoactive intestinal peptide (VIP) - [1.0 μM]
Reference Compound: Vasoactive intestinal peptide (VIP)
Positive Control: Vasoactive intestinal peptide (VIP)
Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 5 mM MgCl$_2$, 1% BSA and 100 μg/ml bacitracin at 37°C for 60 minutes. The reaction is terminated by filtration onto glass fiber filters. Radioactivity trapped onto the filters in determined and compared to control values in order to ascertain any interactions of test compound with the VIP binding site.

Literature Reference: Ogawa, N., et al. Properties and Distribution of Vasoactive Intestinal Polypeptide Receptors in the Rat Brain. *Peptides* **6(1):** 103-109 (1985) with modifications.

Korman, L.Y., et al. Distribution of VIP and Substance P Receptors in Human Colon and Small Intestine. *Digestive Diseases & Sciences.* **34(7):** 1100-1108 (1989).

VASOPRESSIN$_{1A}$
BINDING ASSAY

Reference Compounds	Ki (nM)
■ Arg^8-Vasopressin (AVP)	4.0

Assay Characteristics:

K$_D$ (binding affinity):	1.1 nM
B$_{max}$ (receptor number):	370 fmol/mg protein

Materials and Methods:

Receptor Source:	Human platelets
Radioligand:	[^{3}H]d(CH$_2$)$_5$TyrMe-AVP$_1$ antagonist (40-87 Ci/mmol)
	Final ligand concentration - [0.5 nM]
Non-specific Determinant:	Arg^8-Vasopressin (AVP) - [500 nM]
Reference Compound:	Arg^8-Vasopressin (AVP)
Positive Control:	Arg^8-Vasopressin (AVP)
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl (pH 7.4 at 25°C) containing 0.005% bacitracin, 0.005% soybean trypsin inhibitor, and 0.2% BSA for 90 minutes at 25°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the vasopressin$_{1A}$ binding site.

Literature Reference:	Dorsa, D., Petracca, F., Baskin, D. and Cornett, L. Localization and Characterization of Vasopressin-Binding Sites in the Amygdala of the Rat Brain. *Jrnl. Neuroscience.* **4(7):** 1764-1770 (1984) with modifications.
	Vittet, D., Rondot, A., Cantau, J.M., and Chevillard, C. Nature and Properties of Human Plateley Vasopressin Receptors. *Jrnl. Biochem.* **233:** 631-635 (1986) with modifications.

VASOPRESSIN₁
BINDING ASSAY
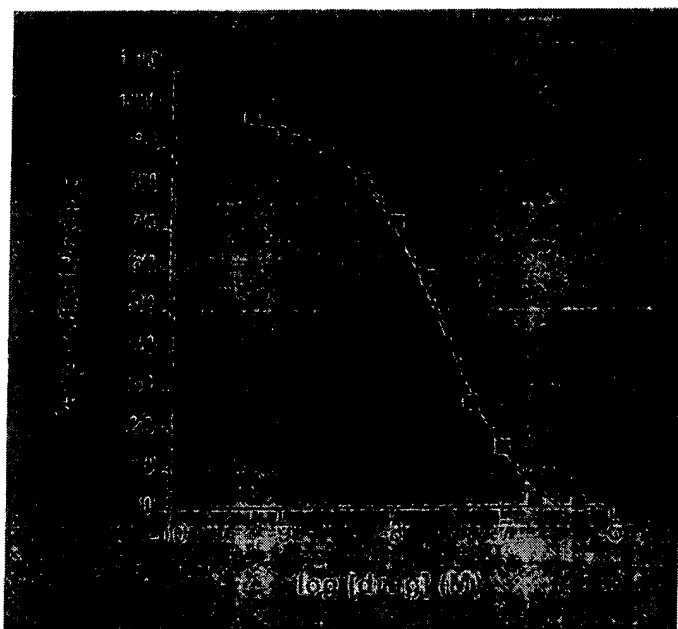
Reference Compounds	Ki (nM)
■ [Arg⁸]-Vasopressin	3.3
[Lsy⁸]-Vasopressin	16.4
[Phe², Ile³, Orn⁸]-Vasopressin	24.1
DDAVP	524.0
Oxytocin	4,480.0

Assay Characteristics:

K_D (binding affinity):   0.3 nM
B_{max} (receptor number):   25 fmol/mg tissue (wet weight)
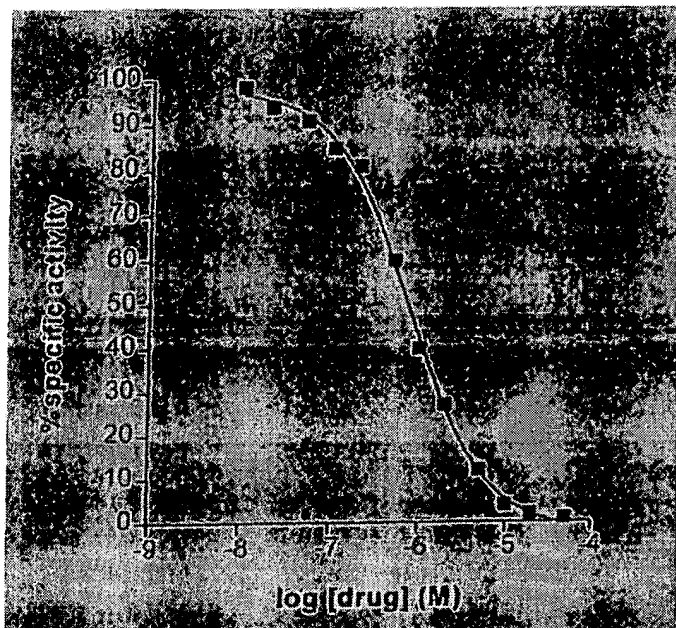
Materials and Methods:

Receptor Source: Rat liver membranes
Radioligand: [³H]phenylalanyl-3,4,5-vasopressin V-1 antagonist (40-87 Ci/mmol)
Final ligand concentration - [0.5 nM]
Non-specific Determinant: Arg⁸-Vasopressin (AVP) - [10.0 uM]
Reference Compound: Arg⁸-Vasopressin (AVP)
Positive Control: Arg⁸-Vasopressin (AVP)
Incubation Conditions: Reactions are carried out in 50 mM HEPES (pH 7.4) for 90 minutes at 0-4°C. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the vasopressin₁ binding site.

Literature Reference: Dorsa, D., Petracca, F., Baskin, D. and Cornett, L. Localization and Characterization of Vasopressin-Binding Sites in the Amygdala of the Rat Brain. *Jrnl. Neuroscience.* **4(7):** 1764-1770 (1984) with modifications.

Gopalakrishnan, V., Triggle, C.R., Sulakhe, P.V. and McNeill, J.R. Characterization of a Specified, High Affinity [³H]Arg⁸ Vasopressin Binding Site on Liver Microsomes from Different Strains of Rat and the Role of Magnesium. *Endocrin.* **118(3):** 990-997 (1986).

ACETYLCHOLINESTERASE
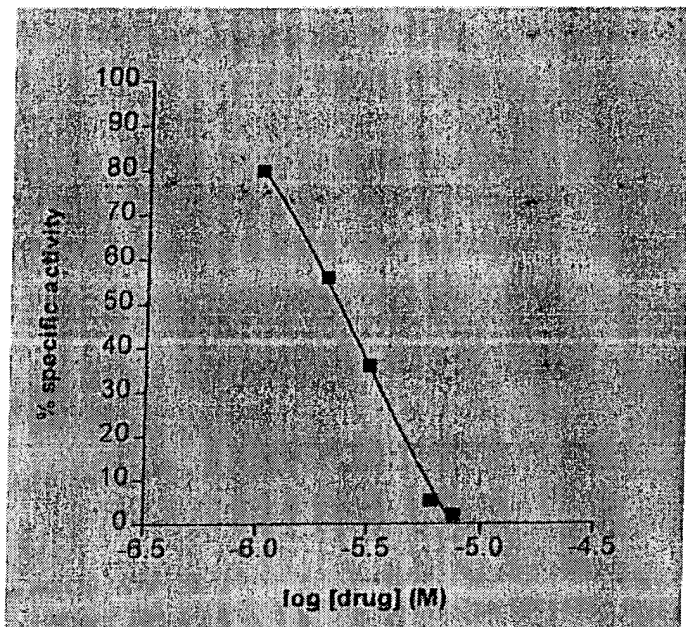## ENZYME ASSAY

Materials and Methods:

Enzyme Source:	Rat brain membranes
Substrate:	Acetylthiocholine
Non-specific Activity:	Physostigmine (Eserine) - [10 μM]
Reference Compound:	Physostigmine (Eserine)
Positive Control:	Physostigmine (Eserine)
Reaction:	Acetylthiocholine → Acetate + Thiocholine
	Thiocholine + DTNB (Ellman's Reagent) → ThiocholineDTNB
Incubation Conditions:	Reactions are carried out in 100 mM KPO$_4$ (pH 8.0) containing 20 μM quinine sulfate and 0.5 mM dinitrothiobenzoic acid. Enzyme is then added and incubated for 15 minutes at 25°C. Enzyme activity is determined spectrophotometrically and samples are compared to control values in order to ascertain any interactions of test compound with the acetylcholinesterase enzyme.

Literature Reference:

G. Ellman, K. Courtney, V. Andres, and R. Featherstone. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochemical Pharmacology.* **7**: 88-95 (1961).

ELASTASE (HUMAN)
## ENZYME ASSAY

Reference Compounds	IC50 (μM)
■ Ursolate	3.2

Materials and Methods:

Enzyme Source:	Human Neutrophils
Substrate:	MeO-Suc-Ala-Ala-Pro-Val-p-Nitroanilide
Non-specific Activity:	Ursolic Acid - [60 μM]
Reference Compound:	Ursolic Acid
Positive Control:	Ursolic Acid
Reaction:	MeO-Suc-Ala-Ala-Pro-Val-pNA → MeO-Suc-Ala-Ala-Pro-Val + pNA
Incubation Conditions:	Reactions are carried out in PBS (pH 7.2). Enzyme is incubated with test/control samples for 30 minutes at R.T. Substrate is then added and incubated for 60 minutes at R.T. Enzyme activity is determined spectrophotometrically and samples are compared to control values in order to ascertain any interactions of test compound with the elastase enzyme.

Literature Reference:

Safahi, H., et. al., Inhibition by Boswellic Acids of Human Neutrophil Elastase. *JPET*. 281:460-463 (1997).

HIV REVERSE TRANSCRIPTASE
ENZYME ASSAY
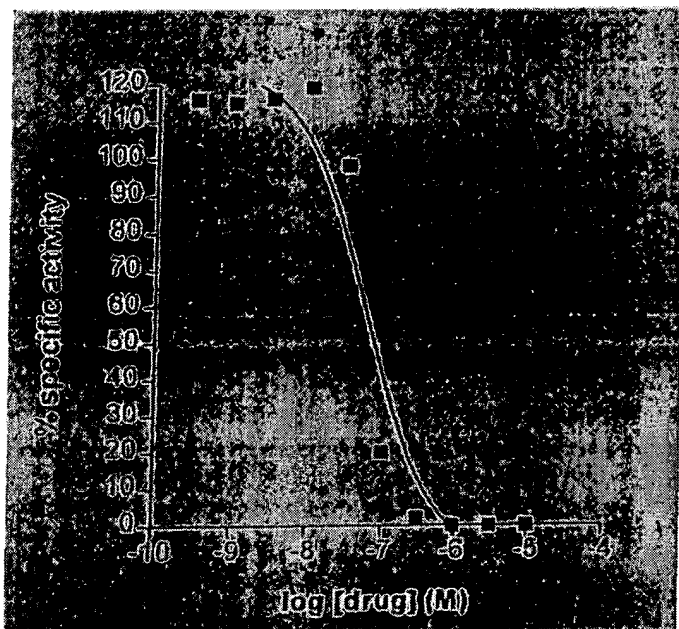
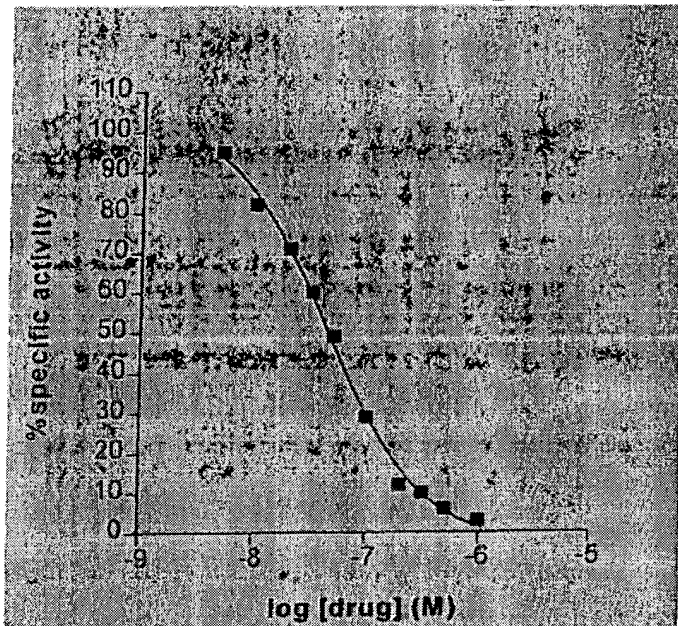| Reference Compound	IC50 (nM)
■ Aurintricarboxylic acid	86.0
3'-Azido-3'-deoxythymidine (AZT)	1,000,000

Materials and Methods:

Enzyme Source:	Recombinant HIV reverse transcriptase expressed in E. coli
Donor Substrate:	[^{3}H] TTP (90-130 Ci/mmol)
Acceptor Substrate:	Biotinylated DNA (17mer) annealed to synthetic random sequence RNA (50mer)
Non-specific Activity:	Determined in the absence of enzyme
Reference Compound:	Aurintricarboxylic acid
Positive Control:	Aurintricarboxylic acid
Reaction:	Catalysis of DNA in the 5' → 3' direction in the presence of RNA template and DNA primer
Incubation Conditions:	Reactions are carried out in 50 mM TRIS-HCl, 80 mM KCl, 10 mM MgCl$_2$, 10 mM DTT, 0.05% w/v nonidet P40 (pH 8.0 at 25°C) for 30 minutes at 37°C. The reaction is stopped by the addition of 0.5 mM EDTA. Radioactive product compared to control values in order to ascertain any interactions of test compounds with the HIV reverse transcriptase enzyme.

Literature Reference:	Reardon, J.E. & Miller, W.H. Human Immunodeficiency Virus Reverse Transcriptase - Substrate and Inhibitor Kinetics with Thymidine 5'-triphosphate & 3'-azido-3'-deoxythymidine 5'-triphosphate. *Jrnl. Biol.l Chem.* **265**: 20302-20307 (1990).

MONOAMINE OXIDASE B (MAO$_B$)
ENZYME ASSAY

Reference Compounds	IC$_{50}$ (nM)
■ RO 166491	40.0
R(-)-Deprenyl	10.0
Clorgyline	2000
Imipramine	53,000
RO 41-1049	>100,000

Materials and Methods:

Enzyme Source:	Rat liver mitochondrial membranes
Substrate:	[^{14}C] Phenylethylamine (0.056 Ci/mmol)
Non-specific Activity:	Ro 166491 - [1.0 µM]
Reference Compound:	Ro 166491
Positive Control:	Ro 166491
Reaction:	[^{14}C] Phenylethylamine → [^{14}C] Phenylacetaldehyde + NH$_4$$^+$
Incubation Conditions:	Enzyme source is preincubated with reference, test, and subtype selective blocker (300 nM clorgyline) for 60 minutes at 37°C in 100 mM KHPO$_4$ (pH 7.2). Substrate is then added and incubated for 7 minutes. The reaction is stopped by the addition of 0.5 ml of 2M citric acid. Radioactive product is extracted into a toluene/ethyl acetate fluor and compared to control values by scintillation spectrophotometry in order to ascertain any interactions of test compounds with the MAO$_B$ enzyme.

Literature Reference:

S. Otsuka & Y. Kobayashi. A Radioisotopic Assay for Monoamine Oxidase Determinations in Human Plasma. *Biochem. Pharmacol.* **13:** 995-1006 (1964) with modifications.

PLASMA ESTERASE (HUMAN)
ENZYME ASSAY
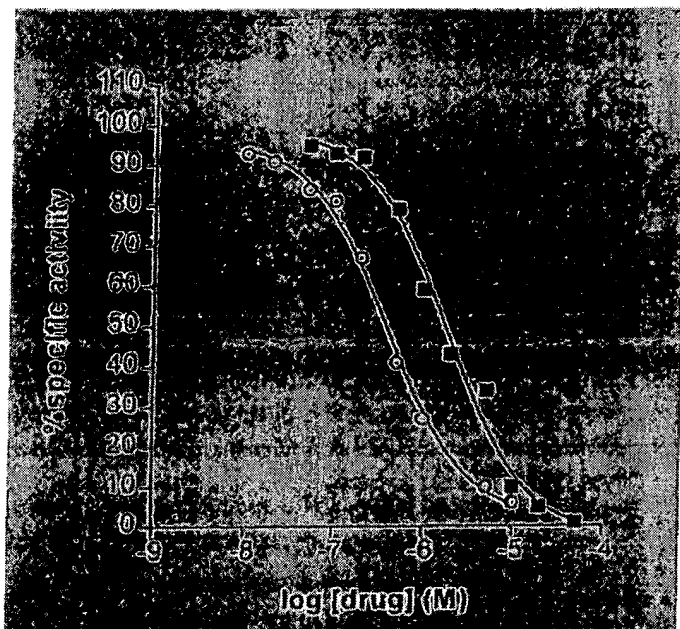
Reference Compounds	IC50 (uM)
■ Eserine	1.4
o Quinidine	0.4

Materials and Methods:

Enzyme Source:	Human Plasma
Substrate:	Acetylthiocholine
Non-specific Activity:	Physostigmine (Eserine) - [10 μM]
Reference Compound:	Physostigmine (Eserine)
Positive Control:	Physostigmine (Eserine)
Reaction:	Acetylthiocholine → Acetate + Thiocholine
	Thiocholine + DTNB (Ellman's Reagent) → ThiocholineDTNB
Incubation Conditions:	Reactions are carried out in 100 mM KPO$_4$ (pH 8.0) containing 0.5 mM dinitrothiobenzoic acid. Enzyme is then added and incubated for 15 minutes at 25°C. Enzyme activity is determined spectrophotometrically and samples are compared to control values in order to ascertain any interactions of test compound with the plasma esterase enzymes.
Literature Reference:	G. Ellman, K. Courtney, V. Andres, and R. Featherstone. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochemical Pharmacology.* **7**: 88-95 (1961).

MONOAMINE OXIDASE A (MAO$_A$)
ENZYME ASSAY
RAT BRAIN
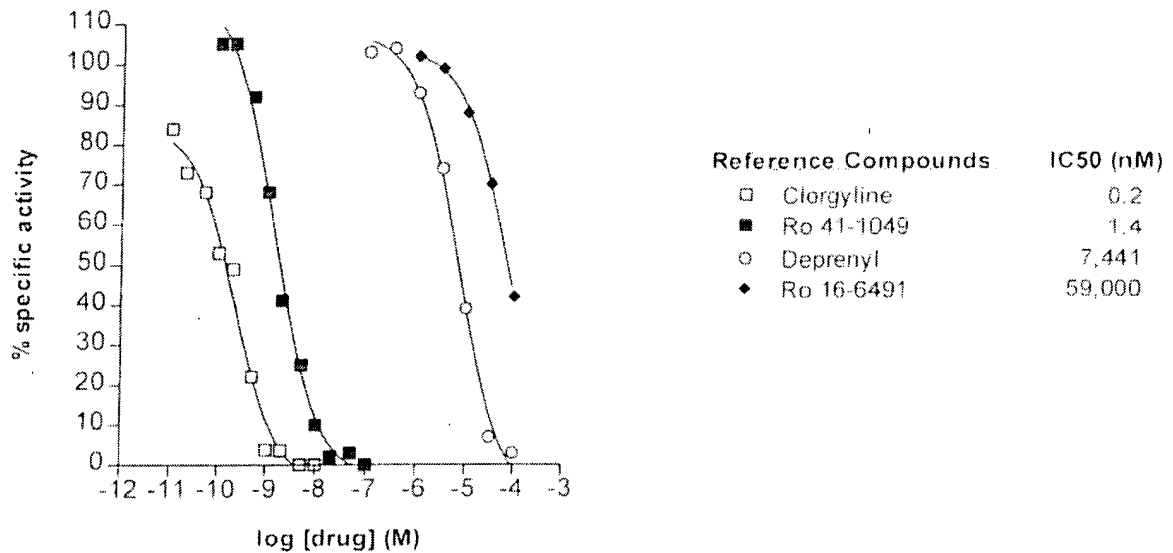
Reference Compounds	IC50 (nM)
□ Clorgyline	0.2
■ Ro 41-1049	1.4
○ Deprenyl	7,441
◆ Ro 16-6491	59,000

Assay Characteristics:

   Degree of Specific Activity: 90 % (Non-specific activity determined using 1.0 μM RO 41-1049)
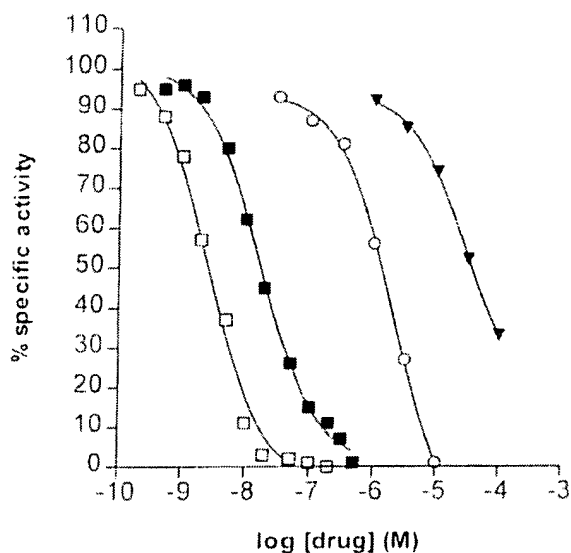
Materials and Methods:

   Enzyme Source: Rat brain
   Substrate: [^{14}C] Serotonin (45-60 Ci/mmol)
   Reference Compound: RO 41- 1049
   Positive Control: RO 41- 1049
   Incubation Conditions: Enzyme source is preincubated with reference, test, and subtype
                            selective blocker (100 nM deprenyl) for 60 minutes at 37°C in 50
                            mM KPO$_4$ containing 50 μM EDTA and 10 μM dithiothreitol (pH 7.2
                            at 25ºC).   Substrate is then added and incubated for 30 minutes.
                            The reaction is stopped by the addition of 0.5 ml of 1-2M citric acid.
                            Radioactive product is extracted into a xylene/ethyl acetate fluor
                            and compared to control values by scintillation spectrophotometry
                            in order to ascertain any interactions of test compound(s) with
                            MAO$_A$.

Literature Reference: S. Otsuka & Y. Kobayashi.   A Radioisotopic Assay for Monoamine
                            Oxidase Determinations in Human Plasma. *Biochem. Pharmacol.*
                            13: 995-1006 (1964) with modifications

MONOAMINE OXIDASE B (MAO$_B$)
ENZYME ASSAY
RAT BRAIN

Reference Compounds	IC50 (nM)
☐ Deprenyl	2.5
■ Ro 16-6491	16.0
○ Clorgyline	2,100
▼ Ro 41-1049	27,000

Assay Characteristics:

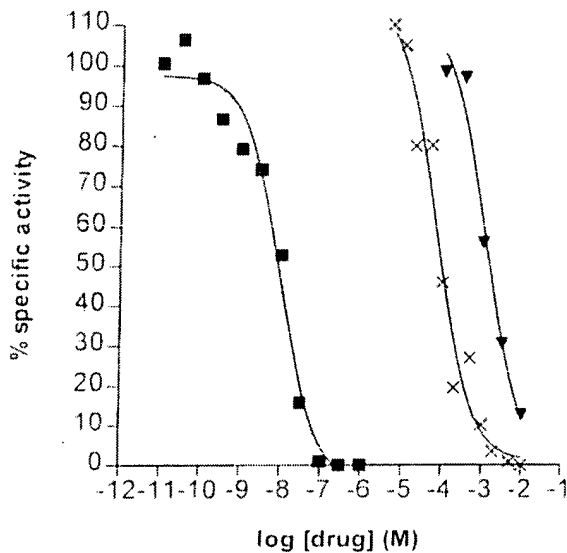   Degree of Specific Activity: 90 % (Non-specific activity determined using 1 μM RO 166491)

Materials and Methods:

Enzyme Source: Rat brain
Substrate: [^{14}C] Phenylethylamine (0.056 Ci/mmol)
Reference Compound: RO 166491
Positive Control: RO 166491
Incubation Conditions: Enzyme source is preincubated with reference, test, and subtype selective blocker (100 nM clorgyline) for 60 minutes at 37°C in 50 mM KHPO$_4$ containing 50 μM EDTA and 10 μM dithiothreitol (pH 7.2 at 25°C). Substrate is then added and incubated for 10 minutes. The reaction is stopped by the addition of 0.5 ml of 1-2M citric acid. Radioactive product is extracted into a xylene/ethyl acetate fluor and compared to control values by scintillation spectrophotometry in order to ascertain any interactions of test compound(s) with the MAO$_B$ enzyme.

Literature Reference: S. Otsuka & Y. Kobayashi. A Radioisotopic Assay for Monoamine Oxidase Determinations in Human Plasma. *Biochem. Pharmacol.* **13:** 995-1006 (1964) with modifications.

GABA TRANSAMINASE
ENZYME ASSAY

Reference Compound	IC50 (μM)
■ Amino-oxyacetic acid	0.01
× Vigabatrin	95
▼ Baclofen	1,180

Assay Characteristics:

Degree of Specific Activity: 75% (Non-specific activity determined with the absence of tissue)

Materials and Methods:

Enzyme Source: Rat brain

Substrate: [^{14}C] GABA (1.5Ci/mmol)

Reference Compound: amino-oxyacetic acid

Positive Control: amino-oxyacetic acid

Incubation Conditions: Reactions are carried out in 50 mM KPO_4 containing 50 μM EDTA and 10 μM dithiothreitol (pH 7.2 at 25°C) for 30 minutes at 37°C. The reaction is stopped by the addition cold 10 μl 5M. Radioactive product is separated by column chromatography (Dowex 50W-X8). Effluent is collected and compared to control values in order to ascertain any interactions of test compound(s) with GABA transaminase enzyme.

Literature Reference:

McManus, D.J., et. al. Biochem. Pharmacol **43:11**, 2486-2489 (1992).